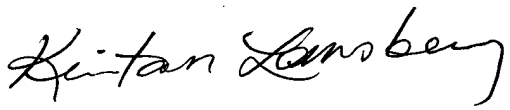
Remarks

The substitute specification has been amended to insert the cross-reference to related application information, specifying that the present application is a national phase application under 35 U.S.C. § 371 of International Application No. PCT/JP98/01728, filed April 15, 1998, which claims priority to Japanese Patent Application No. 97808/1997, filed April 15, 1997, Japanese Patent Application No. 151434/1997, filed June 9, 1997, Japanese Patent Application No. 217897/1997, filed August 12, 1997, Japanese Patent Application No. 224803/1997, filed August 21, 1997 and Japanese Patent Application No. 332241/1997, filed December 2, 1997.

This amendment does raise new issue for further search and consideration as this information was contained in the declaration as previously submitted in the application. This amendment was not presented earlier because the substitute specification was filed on this same date.

If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application or if the Examiner has any questions regarding this application, the Examiner is invited to contact Applicants' undersigned representative at (202) 942-5186.

Respectfully submitted,



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DESCRIPTION

~~NOVEL PROTEIN AND METHOD~~

AN OCIF-BINDING MOLECULE (OBM), NUCLEIC ACID ENCODING, AND PROCESS FOR PRODUCING THE ~~PROTEIN~~SAME

~~Field of Technology~~

TECHNICAL FIELD

The present invention relates to a novel protein (OCIF- binding molecule~~, the protein may be~~; hereinafter ~~called~~ it may be referred to as "OBM"), which binds ~~to~~ osteoclastogenesis inhibitory factor ~~(hereinafter it may be called OCIF)~~, and a production method ~~to produce this protein~~thereof. ~~The~~ In addition, the present invention also relates to DNA ~~encoding~~which ~~this~~encodes the protein, ~~proteins containing the~~a protein having an amino acid sequence encoded by thise DNA, a method for genetically producing the ~~preparation of this~~ protein ~~utilizing genetic engineering techniques~~, and a pharmaceutical compositions comprising thise protein.

The present invention ~~further~~also relates to a methods ~~for~~of screening~~, using this protein and the DNA, substances to control the~~ for: a substance which controls expression of thise protein, ~~substances inhibiting~~a substance which inhibits or ~~regulating~~modifies the biological activity of thise protein, or ~~receptors transducing~~a receptor which binds the ~~signal of~~ protein and transmits the activity thereof, methods of using the protein ~~by interacting with this protein, to~~or the DNA; the substances obtained by ~~the screening~~these methods; and pharmaceutical compositions comprising the obtained substances. In addition, the present invention also relates to an antibody to the protein, a method for the production thereof, a method for measuring the protein with the antibody, and ~~to pharmaceutical compositions which comprise the resulting substances.~~an agent comprising the antibody.

~~The present invention further relates to antibodies against this protein, methods for preparing the antibodies, and pharmaceutical compositions comprising these antibodies.~~

~~Background ArtiH6, 4400, 839, 5~~

BACKGROUND ART

Bone metabolism ~~is dependent~~depends on the overall activity of osteoblasts ~~which~~responsible ~~control~~for bone formation, and osteoclasts ~~which control~~, responsible for bone resorption. ~~Abnormality of~~It is assumed that bone metabolism abnormality is ~~considered to be~~ caused ~~by an imbalance~~due to loss of ~~the~~balance between bone formation and ~~the~~ bone resorption. ~~Osteoporosis~~As diseases involving bone metabolism abnormality, osteoporosis, hypercalcemia, bone Paget~~'~~'s disease, renal osteodystrophy, ~~chronic rheumarthritis, osteoarthristis,~~rheumatoid arthritis and ~~the like~~osteoarthritis are known ~~as diseases accompanying abnormality of bone metabolism~~. ~~Osteoporosis is a typical disease caused by such abnormality of~~ A representative of these bone metabolism abnormality diseases is osteoporosis. This disease ~~is generated~~occurs when bone resorption by osteoclasts exceeds bone formation by osteoblasts~~. The disease~~and is characterized by ~~a~~equal decrease in ~~both the~~ bone calc~~ifi~~ar~~ed~~ous ~~material~~substances and ~~the~~ bone matrix. ~~Although the~~The mechanism for crisis of this disease is not ~~completely elucidated~~yet fully clarified, while it is a disease with pain in bone and bone fracture due to the increased fragility of bone. Along with an increase in the population of aged people, this disease causes ~~aches~~aged people to fracture bone, resulting in ~~bones, makes them fragile, and may result~~confinement in ~~fracturing~~bed. This disease is ~~becoming~~already a social problem ~~because it increases the number of bedridden aged persons as the aged population becomes larger. Development of therapeutic agent~~, so that medicaments for ~~this~~treating the disease ~~is~~are urgently ~~desired.~~needed to be developed. ~~Disease due to a decrease in bone mass~~It is expected ~~to~~that osteopenia due to bone metabolism abnormality can be ~~cured~~treated by ~~suppressing~~stimulating bone formation, inhibiting bone resorption~~, accelerating bone formation~~, or improving the balance between them. That is, bone ~~resorption and formation. Bone~~ formation is expected ~~to~~be ~~increase~~stimulated by ~~accelerating proliferation~~promoting the growth,

differentiation~~, or activation~~ and functions of osteoblasts, which ~~form~~are responsible for bone formation, ~~or by~~ suppressing ~~proliferation,~~the differentiation~~, or activation~~ of osteoclast precursor cells to osteoclasts ~~which resorb~~and maturation thereof, or suppressing osteoclast function such as bone-resorbing activity. ~~In recent years~~At present, ~~strong interest has been directed to~~ hormones, substances of low molecular weight ~~substances,~~ or physiologically active proteins ~~exhibiting~~having such ~~activities, and energetic basic research and development is underway on these subjects~~activity are being studied and developed.

~~Drugs such as~~As agents for treating bone-relating diseases and shortening treatment periods thereof, a calcitonin-containing ~~agents~~formulation, the active-form of vitamin D_3-containing ~~agents~~formulation, hormone ~~agents containing~~ (estradiol, ipriflavone, vitamin K_2) -containing formulation and bisphosphonate~~-compounds have~~-based compound are already ~~been known as drugs to treat and shorten~~clinically available. Furthermore, to develop medicaments with less side effects and excellent effectiveness, clinical trials of the ~~treatment period of diseases related to bone. Clinical tests are in progress on~~ active-form of vitamin D_3 derivatives, estradiol derivatives, and bisphosphonate-based compounds of the second ~~and the~~or third generation ~~to develop therapeutic agents with excellent efficacy and minimal side effects~~have been held.

However, ~~therapies~~since such methods for treatment using these ~~agents were found~~drugs are not necessarily ~~satisfactory~~sufficient in ~~terms of efficacy~~effectiveness and ~~therapeutic~~ results. ~~Development~~ of treatment, novel ~~therapeutic agents which~~medicaments that are safer and ~~with~~have higher ~~efficacy is urgently desired~~effectiveness have been expected to be developed. ~~Some agents~~Moreover, among medicaments used ~~for the~~in treatment of ~~diseases related to~~ bone metabolism diseases, there are those which can be used only ~~limitedly~~for treating a restricted kind of disease due to the~~ir~~ side effects thereof. ~~Furthermore~~In addition, ~~treatments using two or more agents in combination are currently the mainstream in the treatment of diseases related~~at present, to treat bone metabolism diseases such as osteoporosis, treatment with combined use of more than one medicament is currently usual. From such a point of view, ~~development of drugs~~a medicament having different action mechanisms ~~different~~ from

those of the conventional ~~drugs, and exhibiting a~~ones with higher ~~efficacy~~effectiveness and ~~minimal~~less side effects ~~is desired~~have been expected to be developed.

As ~~mentioned~~described above, ~~the~~ cells ~~controlling~~responsible for bone metabolism are osteoblasts and osteoclasts. ~~These cells are~~It is known ~~to have close mutual interactions called~~ "that these cells closely interact with each other, and this phenomenon is regarded as coupling". ~~Specifically~~That is, it has been reported that the differentiation and maturation of osteoclasts are stimulated or suppressed by cytokines ~~such as Interleukins~~, interleukins 1 (IL-1), 3 (IL-3), 6 (IL-6)~~,~~ and 11 (IL-11), granulocyt~~ie~~e -macrophage- colony -stimulating factors (GM-CSF), macrophage- colony -stimulating factors (~~M~~GM-CSF), ~~I~~interferon-~~γ~~ gammas (IFN-~~γ~~γ), tumor necrosis factors ~~α~~α (TNF-~~α~~α)~~, and~~ transforming growth ~~factor-β~~factors β (TGF-~~β~~β)~~,~~ and the like, which are secreted ~~by osteoblastic stromal~~from osteoblast-like stroma cells ~~are known to accelerate or suppress differentiation or maturation of osteoclasts~~ (Raisz: Disorders of Bone and Mineral Metabolism, 287- to 311, 1992; Suda *et al.*: Principles of Bone Biology, 87- to 102, 1996; Suda *et al.*: Endocrine Reviews, 4, 266- to 270, ~~1995~~,1955, Lacey *et al.*: Endocrinology, 186, 2369- to 2376, 1995). It ~~has been reported~~is known that ~~osteoblastic~~osteoblast-like stromal cells play an important role in ~~the~~ differentiation and maturation of osteoclasts~~, as well as in~~ and expression of mature osteoclast ~~functions~~function, such as bone resorption ~~by mature osteoclasts~~, through ~~cell-~~intercellular binding to ~~cell contact with~~ immature ~~osteoclast precursors~~precursor cells of osteoclasts or (mature) osteoclasts. ~~A factor called~~ As a factor involved in osteoclastogenesis by the intercellular binding, a molecule known as osteoclast differentiation factor (ODF) (Suda *et al.*: Endocrine Rev. ~~13~~:13, 66- to 80, 1992; Suda *et al.*: Bone 17, 87S- to 91S, 1995) which is ~~thought to be~~ expressed on the membrane of ~~osteoblastic~~the osteoblast-like stromal ~~cells and involved in the formation of osteoclasts through cell-to-cell contact~~cell is predicted. According to this ~~hypothesis~~assumption, ~~an ODF~~a receptor ~~is present~~for ODF exists in the osteoclast precursor ~~cells of osteoclasts~~cell. However, ~~so far neither the~~ ODF ~~nor~~and the~~is~~ receptor ~~has been~~are not yet either purified or identified. ~~There~~, and there are ~~also~~ no reports ~~relating to~~on their characteristics, action ~~mechanism,~~mechanisms or ~~structure~~nd structures. ~~Thus~~As just described, the mechanism ~~involved in~~for differentiation and maturation of osteoclasts has

not ~~yet~~ been ~~sufficiently elucidated. Clarification~~fully understood yet, and it is expected that full understanding of th~~is~~at mechanism will ~~greatly~~significantly contribute not only to the ~~basic medicine,~~field of experimental medicines but also to ~~the development~~developments of novel ~~drugs~~agents for ~~the treatment of diseases associated with~~treating bone metabolism abnormality ~~of bone metabolism~~, based on the novel action mechanism.

~~The~~Under the circumstances, the present inventors have ~~conducted~~made ~~extensive~~intensive studies ~~in view of this situation~~ and ~~discovered an~~found osteoclastogenesis inhibitory factors (OCIF) in ~~a~~the culture ~~broth~~solution of human ~~embryonic~~fetal lung ~~fibroblast,~~fibroblasts IMR-90 (ATCC ~~Deposition No.~~ CCL186) (WO 96/26217).

~~The~~Then, the present inventors ~~have been successful~~succeeded in DNA cloning ~~DNA encoding~~of OCIF, production of a recombinant OCIF ~~in~~using an animal cell~~s~~, and confirmation of *in vivo* ~~pharmaceutical~~medicinal ~~effects~~virtues (bone metabolism improving effect ~~on bone metabolism, etc.~~) of the recombinant OCIF. OCIF is expected ~~to be used~~ as ~~an agent for~~a medicament that has higher effectiveness and causes less side effects than the ~~prevention or treatment of~~conventional one and can prevent and treat diseases ~~related to abnormality of~~associated with bone metabolism~~, with higher efficacy than conventional drugs and less side effects~~ abnormality.

~~Disclosure of the Invention~~DISCLOSURE OF THE INVENTION

The present inventors have intensively searched for the existence of a protein ~~which~~that binds to osteoclastogenesis inhibitory factor ~~(~~OCIF~~) and discovered~~ by using OCIF. As a result, the inventors have found that ~~an~~ OCIF- binding protein is specifically expressed on ~~the~~an ~~osteoblastic~~osteoblast-like stromal cells cultured in the presence of ~~a~~ bone resorption factors such as the active-form of vitamin D_3 and parathyroid hormone (PTH). ~~In addition~~Furthermore, ~~the present inventors have investigated~~as a result of studying the characteristics and physiological functions of ~~this~~ OCIF- binding protein ~~and found that~~, the protein ~~exhibits~~was found to have biological activity ~~of~~as a ~~factor which supports or promotes the~~so-called osteoclast differentiation and maturation ~~from~~factor, associated with differentiation of immature ~~precursors of~~osteoclast precursor cells to osteoclasts~~. These findings have led to the completion of the present invention~~ and

maturation thereof. ~~Further investigation into the protein of the~~The present invention has ~~proven~~been completed based on this finding. Moreover, as a result of further studying the protein of the present invention, the present inventors have found that ~~this~~the novel membrane protein is an important protein ~~controlling~~which leads the differentiation and maturation of ~~osteoclasts from~~ immature ~~precursors of~~osteoclast precursor cells to osteoclasts by osteoblast-like stromal cells in a co-culture system of the ~~osteoblastic~~osteoblast-like stromal cells and spleen cells. The ~~success in~~successful identification ~~and~~, isolation and purification of the protein ~~which functions~~ as a factor ~~supporting or promoting~~which supports and promotes the differentiation and maturation of osteoclasts in the present invention ~~has~~enables ~~enabled~~a screening ~~for~~of a novel ~~medicine useful for abnormality of~~agent for treating bone metabolism abnormality, based on a mechanism ~~of~~for bone metabolism ~~utilizing~~in a living subject, using the protein of the present invention.

~~Accordingly~~Therefore, an object of the present invention is to provide a novel protein (OCIF- binding molecule ~~or~~; OBM), which binds ~~to~~ osteoclastogenesis inhibitory factor (OCIF), and a method ~~to produce this protein~~for the production thereof. Another object of the present invention is to provide DNA ~~encoding this~~which encodes the protein, ~~proteins containing~~a protein having an amino acid sequence encoded by this DNA, a method for genetically producing this protein ~~utilizing genetic engineering techniques~~, and a pharmaceutical compositions comprising this protein. ~~A~~ Furthermore, ~~further~~another object of the present invention is to provide ~~methods~~an agent for preventing and/or treating bone metabolism abnormality comprising the protein. Moreover, another object of the present invention is to provide: a method of screening ~~substances~~for: a substance which controls expression of ~~this protein using this~~ the protein ~~and the DNA~~, ~~substances inhibiting~~a substance which inhibits or ~~regulating~~modifies the biological activity of this protein, ~~receptors transducing~~or a receptor which binds the ~~action~~protein and transmits the activity of the protein ~~by binding to~~; a method of using the protein~~, substances~~ and DNA thereof; a substance obtained by the~~at screening,~~method; and pharmaceutical compositions ~~which comprises these~~ comprising the obtained substances. ~~A still further~~ Furthermore, another object of the present invention is to provide ~~antibodies against this protein, methods for preparing~~an antibody

to the ~~antibodies~~protein, a method for production thereof, a method for measuring the protein using the antibody, and a medicament (agent; pharmaceutical ~~compositions~~composition) comprising these antibodies.

The protein of the present invention ~~has~~shows the following physicochemical properties and biological activity. That is, (a) ~~Affinity:~~ the protein specifically binds ~~to the~~ osteoclastogenesis inhibitory factor (OCIF) and ~~exhibits~~has high affinity ~~to OCIF~~ (a dissociation constant, a Kd value, on a cell ~~membrane: Kd=~~surface, is not larger than 10^{-9} M ~~or less~~) ; (b) ~~Molecular weight: has~~ the protein shows a molecular weight of ~~approximately~~about 30,000 ~~-~~ to 40,000 ~~when~~as ~~determined~~measured by SDS-polyacrylamide ~~gel~~ electrophoresis ~~(SDS-PAGE)~~ under non-reducing conditions, and shows an apparent molecular weight of ~~approximately~~about 90,000 ~~-~~ to 110,000 when ~~cross-linked~~crosslinked ~~to~~with a monomer ~~form~~-type OCIF; and (c) ~~Biological~~ the protein has an activity: ~~exhibits activity supporting or promoting osteoclast~~ to support and promote the differentiation and maturation of osteoclasts in a co-culture ~~system~~ of ~~the~~ mouse ~~osteoblastic~~osteoblast-like stromal cells and mouse spleen cells in the presence of bone resorption factors such as the active-form of vitamin D_3 and parathyroid hormones (PTH).

~~A~~As a representative *in vitro* culture system for osteoclastogenesis, a co-culture system of ~~ST2, a~~ mouse ~~osteoblastic~~-derived osteoblast-like stromal cell line, ST2, and mouse spleen cells in the presence of the active-form of vitamin D_3 or PTH is well known ~~as a typical in vitro culture system for osteoclast formation~~. The cells ~~expressing~~that express the protein of the present invention can be ~~determined~~obtained by ~~testing~~examining the binding ability of ~~OCIF to~~a mouse ~~osteoblastic~~osteoblast-like stromal cells or mouse spleen cells cultured in the presence or absence of the active-form of vitamin D_3 ~~,~~3 to OCIF. The protein of the present invention is ~~specified~~identified as ~~the~~a protein which is ~~induced~~ specifically induced on ~~the~~an ~~osteoblastic~~osteoblast-like stromal cells cultured in the presence of ~~an osteotropic~~ bone resorption factors such as the active-form of vitamin D_3 or PTH. ~~In addition~~Further, ~~the~~in ~~protein~~consideration of ~~the invention can be specified as a protein exhibiting biological activity supporting or promoting differentiation and maturation of osteoclasts from~~ the following ~~results. That is, the~~facts that osteoclast formation is inhibited ~~dose dependently~~ by ~~the~~ addition of ~~1 to~~

~~40 ng/ml of~~ OCIF to the above~~-mentioned~~ co-culture system in the presence of the active-form of vitamin D_3, ~~the time course of~~in a dose-dependent manner within a range of 1 to 40 ng/ml of OCIF; that there is an intimate correlation between change in expression of the ~~protein of the~~ present ~~invention~~protein induced on the ST2 cells in the presence of the active-form of vitamin D_3 ~~well correlates~~ and the change in osteoclast formation with ~~the~~ the passage of time ~~course of osteoclast formation in the co-culture. In addition,~~; that the amount of ~~protein of~~ the present ~~invention~~protein expressed on a ST2 cells correlatesponds ~~with~~ to the ~~capability~~intensity of ~~the~~an ~~cells~~ability to support ~~the~~ osteoclast formation~~,~~; and ~~the~~that osteoclast formation is completely inhibited by binding of OCIF(s) to the ~~protein of the~~ present ~~invention~~protein on the ST2 cells ~~completely suppresses~~, the protein of the present invention is identified as a protein having biological activity (effect) to support and promote the differentiation and maturation of osteoclasts ~~formation~~.

The affinity of the protein of the present invention ~~to~~for OCIF can be ~~evaluated~~assessed by labeling OCIF and ~~examining~~testing the binding activity of the labeled OCIF to the surface of an animal cell membrane. OCIF can be labeled by a ~~conventional~~commonly used protein-labeling method such as labeling with a radioisotope or fluorescentce labeling. ~~Labeling of tyrosine residues with ^{125}I can be given as a specific~~For instance, an example of labeling ~~of the~~ OCIF with ~~an~~a radioisotope~~. Labeling~~ is ^{125}I labeling at a tyrosine residue, and labeling methods such as ~~i~~Iodogen method, chloramine T method~~, and enzymatic~~ and enzyme method ~~can be utilized.~~ can be employed thereto. The binding ~~of the~~activity of the thus labeled OCIF to the surface ~~membrane~~of ~~of~~an animal cell membrane can be examined in accordance with a commonly used method, and the amount of nonspecific binding can be ~~tested~~measured by ~~a conventional method. The addition~~adding 100 to 400 times excess amounts of unlabeled OCIF to the medium ~~used~~ for the binding ~~assay to a concentration, 100 to 400 times the concentration of labeled OCIF, ensures measurement of non-specific binding~~experiment. The amount of specific binding of OCIF ~~can be~~is calculated by subtracting ~~the amount~~that of ~~non-specific~~the nonspecific binding from ~~the~~that of total ~~amount of~~ binding ~~of the labeled OCIF~~. The affinity ~~of the protein~~(for OCIF) of the present ~~invention~~protein expressed on ~~the~~a cell membrane ~~to OCIF can be evaluated~~is

assessed by chaongducting the amounttest with various amounts of the labeled OCIF and analyzing the amount of the specific binding by Scatchard plot. The determined affinity of the protein of the present invention tofor OCIF is approximatelyabout 100- to 500 pM. TheThus, the protein of the present invention is specifiedidentified byas a protein having such high affinity (the dissociation constant on cell membrane: -, the Kd =value, on a cell membrane is not larger than 10^{-9} M or less) to osteoclastogenesis inhibitory factor (for OCIF). The molecular weight of OBM can be accessedis measured by use of gel filtration chromatography, SDS-PAGE, or the like. To measure the molecular weight more accurately, SDS-PAGE is preferred in order to accurately determine the molecular weight. Thepreferably used, and OBM is specifiedidentified as a protein having a molecular weight of approximatelyabout 40,000 (40,000 ±± 4,000) under reducing conditions.

The protein of the present invention can be obtained from a mouse osteoblasticosteoblast-like stromal cell line, ST2, a mouse preadipocytefat cell line,strain PA6, or human osteoblasticosteoblast-like cell lines, or otherconcentrated osteoblasticosteoblast-like cells selectedobtained from mammalians such as humans, mice, or ratsmouse and rat. As theAnd, substances that are required to induce expression ofexpress the protein of the present invention, osteotropic on these cells may be bone resorption factors such as the active-form of vitamin D_3 (calcitriol), parathyroid hormone (PTH), interleukins (IL)-1, IL-6, IL-11, Ooncostatin M, and leukemia inhibitorycell growth inhibiting factor (LIF) can be given. TheseAs for the amounts of these substances can be added in, it is desirable to use the concentration of 10^{-8} M (active-form of vitamin D_3 andor PTH), in an amount of 10^{-8} M; the IL-11 and the oncostatin M in amounts of 10 ng/ml (IL-11), orand 1 ng/ml (Oncostatin M). -, respectively; and the IL-6 is preferably used at a concentrationin an amount of 20 ng/ml with 500 ng/ml of IL-6 soluble IL-6 receptor. Preferably, confluentIt is preferable to use cells ofobtained by culturing mouse osteoblasticosteoblast-like stromal cell line, ST2, cultured in α-α-MEM medium to whichcontaining 10^{-8} M of the active-form of vitamin D_3, 10^{-7} M of dexamethasone, and 10% fetal bovine fetal serum were added can be usedfor at least one week until the cells become confluent. Theus cultured cells may becan be removed and collected by scraping

~~with~~using a cell scraper or the like. ~~The~~Moreover, the collected cells ~~may~~can be stored at -80~~°~~°C until use.

The protein of the present invention can be purified efficiently from ~~the~~a membrane fraction~~s~~ of the thus collected cells. The membrane fractions can be prepared ~~by~~in accordance with a ~~conventional~~common method ~~which is used to prepare intracellular organella. Various types~~used for fractionation of ~~protease inhibitors may be added to the~~ organelles. As a buffer ~~solution used for the~~ used in preparation of the membrane ~~fractions~~fraction, various protease inhibitors may be preferably added. ~~Examples~~Illustrative examples of ~~the~~ protease inhibitors to be added include serine protease inhibitors, thiol protease inhibitors, and metalloprotease inhibitors~~.~~, such as PMSF, APMSF, EDTA, ~~o~~O-phenanthroline, leupeptine, pepstatin A, aprotinin~~,~~ and a soybean trypsin inhibitor ~~are givens as specific examples~~. ~~A Daunce homogenizer~~To crush the cells, a ~~polytron~~Dounce homogenizer, a polythoron homogenizer, an ultrasonicator or ~~a ultrasonic processor~~the like can be used ~~to homogenize the cells~~. The ~~cell homogenate is~~crushed cells can be suspended in a buffer ~~solution~~ containing 0.5 M ~~of~~ sucrose and centrifuged at 600 X g for 10 minutes ~~at 600 x g,~~so as to separate ~~the~~cell nucle~~us~~i and un~~dic~~ruptshed cells as ~~precipitate~~a precipitated fraction. ~~The supernatant is centrifuged~~After further centrifugation at 150,000 X g for 90 minutes ~~at 150,000 x g to obtain~~, a membrane ~~fractions~~fraction can be obtained as ~~precipitate~~a precipitated fraction. ~~The~~By treating the thus obtained membrane fraction ~~is treated by~~with various ~~types of detergents to efficiently solubilize and extract~~surfactants, the protein of the present invention ~~from~~existing on the cell membrane can be solubilized and extracted, efficiently. ~~Detergents~~For solubilization, various surfactants which are ~~commonly~~conventinally used ~~to solubilize~~in solubilization of cell membrane proteins, such as CHAPS (3-[(3-cholamidopropyl)-dimethylammonio]-1- propanesulfonate), Triton X-100, Nikkol~~,~~ and n-~~octyl-glycoside~~octylglycoside, can be used. ~~Preferably,~~The protein of the present invention is preferably solubilized by adding 0.5% CHAPS ~~is added~~ to the ~~membrane fraction~~protein and agitating the mixture ~~is~~at ~~stirred~~4°C for 2 hours ~~at 4°C to solubilize the protein of the present invention~~. ~~The sample~~ By centrifuging the thus prepared ~~is centrifuged~~ sample at 150,000 ~~x~~X g for 60 minutes ~~to obtain~~, the solubilized membrane fraction can be obtained as a supernatant.

The protein of the present invention can be purified efficiently from the thus obtained solubilized membrane fraction ~~with a~~, using an OCIF-immobilized column, gel~~,~~ or resin ~~coupled with OCIF~~. ~~The~~As ~~immobilized~~the OCIF ~~may~~to be used in the immobilization, that isolated from ~~a~~the culture ~~broth~~solution of human ~~embryonic~~fetal lung fibroblasts, IMR-90, in accordance with a method described in WO 96/26217 or ~~rOCIF prepared using~~that obtained by genetic engineering ~~techniques~~(rOCIF) can be used. This rOCIF can be ~~prepared~~obtained by ~~introducing~~incorporating the corresponding human ~~cDNA, rat or~~ mouse ~~cDNA, or rat~~ cDNA into an expression vector ~~according to~~in accordance with a ~~conventional~~common method, ~~transducing~~expressing the ~~constructed vector in~~rOCIF with animal or insect cells such as CHO cells, BHK cells~~,~~ ~~or~~and Namalwa cells, ~~or in insect cells to produce rOCIF,~~ and then purifying ~~rOCIF~~it. ~~Obtained~~The thus obtained OCIF ~~has~~shows a molecular weight of ~~approximately~~about 60 kDa (monomer ~~form~~ type) ~~or~~and a molecular weight of about 120 kDa (dimer ~~form~~ type). ~~The~~A dimer ~~form~~ type OCIF is ~~preferable for~~preferably used in the immobilization. ~~Given as examples of the gels and resins to which~~As a gel or a resin for immobilizing OCIF ~~is immobilized are~~, ECH ~~Sepharose~~SEPHAROSE® 4B, EAH ~~Sepharose~~SEPHAROSE® 4B, ~~T~~thiopropyl ~~Sepharose~~SEPHAROSE® 6B, CNBr--activated ~~Sepharose~~SEPHAROSE® 4B, activated CH ~~Sepharose~~SEPHAROSE® 4B, ~~E~~epoxy activated ~~Sepharose~~SEPHAROSE® 6B, activated thiol ~~Sepharose~~SEPHAROSE® 4B (~~these are manufactured by~~products of Pharmacia Co., Ltd.), TSK~~k~~gel AF-~~E~~epoxy ~~Toyopal~~TOYOPAL 650, TSK~~k~~gel AF-~~A~~amino ~~Toyopal~~TOYOPAL 650, TSK~~k~~gel AF-~~F~~formyl ~~Toyopal~~TOYOPAL 650, TSK~~k~~gel AF-~~C~~carboxy ~~Toyopal~~TOYOPAL 650, TSK~~k~~gel AF-Tresyl ~~Toyopal~~TOYOPAL 650 (~~these are manufactured by Tosoh Corporation~~products of Toso Co., Ltd.), ~~A~~amino-~~Cellulofine,~~CELLULOFINE™ ~~C~~carboxy-~~Cellulofine~~CELLULOFINE™, FMP activated ~~Cellulofine~~CELLULOFINE™, ~~Formyl-Cellulofine~~formyl-CELLULOFINE™ (~~these are manufactured by Seikagaku Kogyo~~products of Sei Kagaku Kougyo Co., Ltd.), ~~Affigel~~ AFFIGEL 10, ~~Affigel~~AFFIGEL ~~15,~~15 and ~~Affiprep~~AFFIPREP 10 (~~these~~products of Bio-Rad Co., Ltd.) are ~~manufactured by BioRad Co.)~~available. ~~As columns to which~~Furthermore, as a column for immobilizing OCIF ~~is immobilized~~, ~~HiTrap~~a HITRAP® NHS-activated column (Pharmacia Co., Ltd.), TSKgel Tresyl-5PW (~~Tosoh~~

~~Corporation~~Toso Co., Ltd.)~~, etc.~~ or the like can be ~~given~~used. As a specific example of ~~the~~a method for immobilizing OCIF ~~to a HiTrap~~with the HITRAP® NHS-activated column (1 ml, Pharmacia Co., Ltd.), the following method ~~can be given~~is presented. ~~Specifically~~That is, 1 ml of 0.2 M $NaHCO_3$/0.5 M NaCl ~~solution~~ (pH 8.3) solution containing 13.0 mg of OCIF is ~~injected~~applied to the column and allowed to ~~perform~~undergo a coupling reaction at room temperature for 30 minutes. Then, after 0.5 M ethanolamine/0.5 M NaCl (pH 8.3) and 0.1 M acetic acid/0.5 M NaCl (pH 4.0) are ~~sequentially~~ applied ~~to the column. Then, the column is again washed with~~respectively, 0.5 M ethanolamine/0.5 M NaCl (pH 8.3) is applied again, and then the column is ~~allowed~~left to stand ~~for one hour~~ at room temperature for 1 hour so as to ~~block~~inactivate excess active groups. ~~The~~Thereafter, the column is ~~sequentially~~ washed twice with 0.5 M ethanolamine/0.5 M NaCl (pH 8.3) and 0.1 M acetic acid/0.5 M NaCl (pH 4.0), and then ~~washed~~replaced with 50 mM Tris/1 M NaCl/~~0.1~~0.1% CHAPS ~~solution~~buffer (pH 7.5)~~, thereby obtaining a~~. Finally, an OCIF-immobilized column can be prepared. Using the prepared OCIF-immobilized column~~. The~~, gel or resin, the protein of the present invention can be ~~efficiently~~ purified ~~by a OCIF-immobilized column prepared in this manner, or an OCIF-immobilized gel or resin~~efficiently.~~It is desirable to add~~ To prevent the proteolysis of the protein of the present invention, the above various ~~above-mentioned~~ protease inhibitors may also be added to the buffer solutions used ~~for the~~in purification ~~of the protein to suppress degradation of the protein of the present invention~~. ~~The protein of the present invention can be purified by loading~~After applying the above-~~mentioned~~ solubilized membrane fraction ~~on~~to ~~the~~an OCIF-immobilized column or ~~by~~ mixing the solubilized membrane fraction with ~~the~~an OCIF-immobilized gel or resin~~,~~ and ~~eluting~~subsequently stirring the mixture so as to cause the fraction to be adsorbed, the protein of the present invention can be eluted from the OCIF-immobilized column, gel~~,~~ or resin ~~with~~using an acid, various protein -denaturating agents, a cacodylate buffer~~,~~ ~~and~~or the like. ~~It is desirable to use an acid for elution and to neutralize immediately after elution to~~To minimize denaturation of the protein of the present invention, it is preferable to neutralize the eluate immediately using a base. As ~~the acidic~~an acid buffer solution used for elution, 0.1 M glycine-hydrochloric acid buffer solution (pH 3.0), 0.1 M glycine-

hydrochloric acid buffer solution (pH 2.0)~~, and~~ 0.1 M sodium citrate buffer solution (pH 2.0)~~, and the like~~ can be ~~given~~used, for example.

The purified protein of the present invention can be further purified by ~~conventional~~use of a method which is conventionally employed in purification ~~methods used for purification of various~~of proteins from biological ~~materials and by~~samples, through various purification ~~methods utilizing~~operations taking advantages of the physicochemical properties of thi~~s~~e protein of the present invention. To concentrate ~~solutions containing~~a solution of the protein of the present invention, ~~conventional techniques~~a method which is conventionally used in ~~the~~protein purification process ~~for proteins such as ultra filtration~~, e.g., ultrafiltration, freeze -drying~~,~~ and salting-out, can be used. ~~Ultra filtration~~Preferably, ultrafiltration based on centrifugation with ~~Centricon~~-CENTRICON®-10 (~~BioRad~~Amicon Co.)~~, for example,~~ and the like is ~~preferably~~ used. ~~As~~Furthermore, as a means ~~for the purification~~of purificiation, various ~~techniques~~methods conventionally ~~utilized for the~~used in protein purification ~~of proteins, such as~~using ion exchange chromatography, gel filtration chromatography, hydrophobic chromatography, reverse~~d~~ phase chromatography,~~ and~~ preparative electrophoresis~~, are~~ and the like can be used in combination. More specifically, ~~it is possible to purify~~ the protein of the present invention can be purified by ~~a~~concombined ~~combination~~use of gel filtration chromatography with ~~Superose~~ SUPEROSE®-12 column (Pharmacia Co., Ltd.) and the like and reverse phase chromatography. ~~To detect~~Moreover, the protein of the present invention ~~in~~during the purification process~~, the binding~~ can be detected by analyzing activity ~~of the protein of the present invention~~ to bind the immobilized OCIF ~~is examined~~ or ~~the material bound to the immobilized OCIF is~~by immuno ~~precipitated~~precipitation of OCIF-binding substances with an anti-OCIF antibody ~~and analyzed~~followed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) analysis.

The thus obtained protein of the present invention is useful, due to its activity, as ~~an agent~~medicaments, e.g., as agents for treating ~~diseases caused by abnormality of~~ bone metabolism abnormality such as osteopetrosis, or ~~as a reagent for research~~experimental and ~~diagnosis of these diseases~~diagnostic reagents.

~~The~~Furthermore, the present invention ~~further~~relates ~~provides~~to DNA ~~encoding~~which encodes a novel protein (OCIF- binding molecule ~~or~~; OBM) which binds

~~to~~ osteoclastogenesis inhibitory factor (OCIF), ~~proteins containing the~~a protein having an amino acid sequence encoded by thi~~s~~e DNA, a method for ~~the preparation of this protein~~genetically producing a protein which specifically binds OCIF by use of the ~~genetic engineering technique~~protein, and ~~pharmaceutical compositions~~an agent for treating bone metabolism abnormality comprising thi~~s~~e protein. ~~Furthermore~~In addition, the present invention ~~provides methods~~relates to a method for screening ~~substances to regulate~~a substance which controls expression of OBM, a method for screening ~~substances inhibiting or modifying the biological activity of~~a substance which binds OBM and inhibits or modifies an effect thereof, ~~or~~ a method for screening ~~receptors transducing the action of~~a receptor which binds OBM ~~by binding to OBM~~and transmits an effect thereof, and pharmaceutical compositions ~~which comprises~~ comprising a substances obtained as a result of ~~the~~these methods for screening.

The novel protein OBM ~~which is~~ encoded by the DNA of the present invention ~~has~~shows the following physicochemical properties and biological activity. That is, (a) ~~binds~~ the protein specifically ~~to~~binds osteoclastogenesis inhibitory factor (OCIF), (b) ~~has~~ the protein shows a molecular weight of ~~approximately~~about 40,000 (~~*~~± 4,000) ~~when~~as ~~determined~~measured by SDS-PAGE under reducing conditions, and shows an apparent molecular weight of ~~approximately~~about 90,000~~-~~ to 110,000 when crosslinked ~~to~~with a monomer ~~form~~type OCIF~~,~~; and (c) ~~exhibits~~ the protein has an activity ~~supporting or promoting~~to support and promote differentiation and maturation of osteoclasts.

Human osteoclastogenesis inhibitory factor (OCIF) ~~which~~ is used as a probe ~~to identify~~for assessing the properties of OBM in identification of the DNA encoding ~~OBM, the~~ OCIF- binding molecule OBM of the present invention, and ~~to evaluate properties of OBM~~ can be isolated from ~~a~~the culture ~~broth~~solution of ~~a~~ human ~~embryonic~~fetal lung ~~fibroblast cell line~~fibroblasts, IMR-90, ~~according to~~in accordance with WO ~~No.~~ 96/26217. ~~Recombinant~~For isolation and identification of the DNA encoding OBM, recombinant human OCIF, recombinant mouse OCIF, recombinant rat OCIF~~,~~ and the like can also be used ~~for the isolation and identification of the DNA coding OBM~~. The~~i~~se recombinant OCIF ~~proteins~~ can be ~~produced~~obtained by ~~inserting~~incorporating the corresponding DNA ~~fragments encoding these proteins~~ into an expression ~~vectors according to conventional methods~~vector in accordance with a commonly used method,

subsequently expressing ~~in~~OCIF with animal or insect cells such as CHO cells, BHK cells~~,~~ ~~or~~and Namalwa cells, ~~or in insect cells,~~ and then purifying ~~them~~it.

~~As a method~~Methods for ~~isolating~~cloning a cDNA ~~encoding a~~which encodes the target protein (cDNA cloning) include, ~~the~~a method comprising ~~determination~~the steps of determining a partial amino acid sequence of the protein and ~~isolation of~~isolating the target cDNA by hybridization ~~utilizing the~~based on a nucleotide sequence corresponding to the amino acid sequence ~~can be employed~~. Another ~~available~~ method~~, even in~~ comprises the ~~case where~~steps of constructing a cDNA library with an expression vector, regardless of whether or not the amino acid sequence of the protein is ~~not~~ known, ~~comprises constructing a cDNA library in a expression vector,~~subsequently introducing ~~the cDNA~~it into cells, and then screening for the presence and absence of expression of the target protein ~~to~~and isolate~~ing~~ the ~~objective~~desired cDNA (~~expression cloning method,~~ D' Andrea *et al.*: Cell 57, 277- to 285, 1989; Fukunaga *et al.*: Cell 61, 341- to 350, 1990) (expression cloning method). In the expression cloning method, ~~suitable host cells such as bacteria~~bacterial, yeast, animal cells~~,~~ and the like are selected ~~depending on the objective. In many cases, animal cells are selected~~and used as ~~the~~ host cells ~~for~~according to the purpose. For cloning a cDNA ~~encoding a~~ which encodes a protein ~~such as the protein of the present invention which is~~ considered to ~~be~~ present ~~in~~on the surface of animal cell membrane ~~surface. Normally~~as in the present invention, ~~host~~animal cells ~~showing~~are often used as hosts. Furthermore, hosts with high efficiency for introducing DNA ~~transfection~~ and ~~achieving expression of~~expressing the introduced DNA ~~at high levels~~ are ~~selected~~conventionally used. One of ~~such animal~~the cells having such characteristics is ~~the~~a monkey kidney ~~cells~~cell line, COS-7, used in the present invention. ~~Because~~Since SV40 large T antigen is expressed in ~~the~~ COS-7 cells, plasmids having ~~a plasmid which has a replicator of~~ SV40 ~~can be~~origin of replication are present in the cell as ~~an~~multicopy episome ~~of multiple copies in the cell, so that a high level of~~whereby higher expression ~~is~~than usual can be expected. ~~In addition~~Moreover, ~~because expression of a target protein by COS-7 cells reaches a~~since the maximum expression level is reached within a few days after the introduction of DNA, ~~the cell is~~COS-7 cells are suitable for ~~rapid~~quick screening. ~~A~~In combination ~~of this host cell~~ with a plasmid ~~capable~~suitable ~~of~~for high expression ~~ensures gene expression of~~, this host cell enables an

extremely high level of gene expression. The promoter is a factor ~~exhibiting the greatest influence on the expression of a gene on~~ a plasmid ~~is~~which has the most significant effects on the amount of gene expression. As a promoter~~. Promoters such as SRα~~ suitable for high level of expression, SRα promoter and cytomegalovirus-derived promoters are often used ~~as high expression promoters~~. ~~To~~Screening ~~screen~~methods for cloning the cDNA ~~encoding~~of ~~a~~the membrane protein ~~by the~~include expression cloning ~~strategy, screening procedures such as~~ binding method, panning method~~,~~ ~~or~~and film emulsion method ~~are used~~.

The present invention relates to DNA ~~encoding~~, which encodes the protein ~~(OBM)~~ which specifically binds ~~to~~ OCIF~~, isolated~~ (OBM), obtained by ~~the~~a combination of the expression cloning ~~strategy~~method and ~~the screening by~~ the binding method, ~~to~~ the protein expressed ~~protein~~ therewith, and ~~to~~a screening of ~~physiologically~~a biologically active substances ~~using~~with the DNA or the ~~expressed~~ protein. OBM encoded by the DNA of the present invention can be detected by labeling OCIF and ~~testing~~subsequently examining the binding activity of the labeled OCIF to ~~membrane~~the surface of an animal cell membrane. OCIF can be labeled by a conventional method for labeling ~~method~~protein such as labeling with a radioisotope ~~labeling method~~ or fluorescent~~c~~e labeling ~~method which~~. An example of labeling OCIF with radioisotope is ~~used for~~ ^{125}I labeling ~~common proteins. Labeling~~at tyrosine residues ~~by~~ ~~^{125}I can be given as a~~, and specific ~~example of~~ labeling ~~OCIF with a radioisotope. Labeling~~ methods ~~such as the iodogene~~include Iodogen method, chloramine T method~~,~~ and enzymat~~ic~~e method ~~can be utilized~~. The binding activity of thus labeled OCIF to the surface of an animal cell membrane ~~surface~~ can be ~~tested~~assessed in accordance with a commonly used method. Furthermore, an amount of nonspecific binding can be measured by ~~conventional methods. The addition~~adding 100 to 400 times excess amount of unlabeled OCIF to the medium ~~used~~ for ~~the test to~~ a ~~concentration, 100 to 400 times the concentration of labeled OCIF, enables quantification of the amount of non-specific~~ binding experiment. The amount of specific binding of OCIF ~~can be~~is calculated by subtracting that of the ~~amount of non-specific~~nonspecific binding from that of the total ~~amount of~~ binding ~~of the labeled OCIF~~.

~~The present inventors~~ Based on an assume~~d~~ption that ~~there is interaction between the~~a factor, which is involved in differentiation of osteoclasts ~~and OCIF. Based on this assumption, to isolate the protein to which recombinant~~interacts with OCIF ~~binds~~, the inventors have screened ~~the~~an expression library prepared from the mRNA of a mouse ~~osteoblastic~~osteoblast-like stromal cell line, ST2, ~~according to~~with recombinant OCIF in accordance with the following method in order to separate the protein which binds OCIF. ~~Specifically,~~ DNA synthesized ~~using~~from the mRNA of ST2 ~~mRNA~~cells was inserted into an expression vector for an animal ~~cells~~cell, and ~~the vector with the insert was introduced~~they were transduced (transfected) into COS-7 monkey kidney ~~COS-7~~ cells. ~~The objective~~Using ^{125}I-labeled OCIF as a probe, the target protein expressed on the COS-7 cells was screened ~~using OCIF labeled with ^{125}I as a probe~~. As a result, DNA ~~encoding~~which encodes the protein ~~which binds~~that specifically ~~to~~binds OCIF ~~was isolated. The~~could be separated, and then the nucleotide sequence of the DNA ~~encoding~~which encodes this OCIF- binding molecule (OCIF- binding molecule; OBM) was ~~then~~ determined. ~~Moreover~~Furthermore, it has been found that OBM encoded by thi~~s~~e DNA ~~was found to bind~~ strongly and specifically ~~and strongly to~~binds OCIF~~,~~ on the cell membrane.

~~Comparatively~~An example of DNA hybridization under relatively mild conditions ~~for hybridization of DNA~~ in the present invention ~~are the conditions, for example, wherein~~is that after DNA is transferred to a nylon membrane and ~~immobilized thereto according to conventional methods and~~fixed in accordance with a common method, it is hybridized ~~in a buffer solution for~~with a radio-labeled DNA as a probe in a hybridization ~~with a probe DNA labeled with an isotope~~buffer at ~~a temperature of~~ 40~~-~~ to 70°C for about 2 hours to overnight, ~~followed by~~ and then wash~~inged in~~ with 0.5 ~~x~~X SSC (0.075 M sodium chloride and 0.0075 M sodium citrate) at 45°C for 10 minutes. ~~Specifically~~More specifically, ~~Highbond~~after DNA is transferred and fixed to a nylon membrane, HYBOND® N (Amersham Co.~~) is used as the nylon membrane to transfer and immobilize DNA thereon~~, Ltd.~~ DNA~~), in accordance with a conventional method, it is ~~then~~ hybridized with a ~~probe DNA~~ ^{32}P-labeled ~~with ^{32}P~~DNA as a probe in ~~a rapid hybridization buffer~~Rapid Hybridization Buffer (Amersham Co., Ltd.) at 65°C for 2

hours, ~~followed by~~ and then washinged with 0.5 ~~x~~X SSC (0.075 M sodium chloride and 0.0075 M sodium citrate) at 45°C for 10 minutes.

~~A~~As a representative *in vitro* culture system for osteoclastogenesis, a co-culture system of mouse-derived ~~osteoblastic~~osteoblast-like stromal ~~cells~~cell line, ST2, and mouse spleen cells in the presence of the active-form of vitamin D_3 or PTH is well known ~~as a typical in vitro culture system for osteoclast formation. The protein~~ OBM of the present invention is ~~specified as the~~identified as a protein which is ~~induced~~ specifically induced on ~~the osteoblastic stromal cells~~an osteoblast-like stroma cell cultured in the presence of ~~an agent which accelerates~~ bone resorption factors such as an active-form of vitamin D_3 ~~or~~and PTH. ~~In addition~~Furthermore, ~~because of the fact that formation of~~since osteoclasts formation is stimulated by ~~the addition of~~adding the protein encoded by the DNA of the present invention to a culture system of mouse spleen cells ~~cultured~~ even in the absence of the active-form of vitamin D_3 or PTH, OBM ~~which is~~ encoded by the DNA of the present invention is considered to be involved in ~~the~~ differentiation and maturation of osteoclasts.

~~Recombinant~~A recombinant OBM can be produced by inserting the DNA of the present invention into an expression vector so as to ~~construct~~prepare a plasmid for expressing OBM, and then introducing and expressing the plasmid ~~into~~in various cells ~~or microorganisms to express recombinant OBM~~and microbial strains. ~~As a host in which recombinant OBM is expressed, mammalian cells such as~~ COS-7, CHO, Namalwa~~, or bacteria such~~ and the like can be used as mammalian hosts cells for expression, and *Escherichia coli (E. coli)* and the like can be used as bacterial host cells for expression. In such a case, the recombinant OBM ~~may~~can be expressed as a membrane-bound ~~form~~ protein using ~~the~~ full length of DNA or as a secreti~~on~~ry ~~form~~type or ~~a~~solubilized-type (soluble ~~form~~type) protein by removing a part of the ~~portion~~DNA encoding ~~the~~a ~~transmembrane~~membrane-binding domain from the full length. The~~us~~ produced recombinant OBM can be purified efficiently ~~purified using a suitable~~in combination ~~of~~with conventional methods used in protein purification ~~methods used for common proteins,~~ such as affinity chromatography using an OCIF-immobilized columns, ion exchange chromatography, ~~and~~ gel filtration chromatography and the like. The thus obtained protein of the present invention is useful, due to its activity, as ~~an~~

~~agent~~medicaments, e.g., as agents for treating ~~diseases caused by abnormality of~~ bone metabolism abnormality such as osteopetrosis ~~or as a reagent for research~~ and ~~diagnosis of such diseases~~as experimental or diagnostic reagents.

The ~~following screening operations can be carried out using the~~ protein OBM encoded by the DNA of the present invention enables: (1) screening of ~~substances~~a substance which ~~regulate~~controls expression of OBM~~,~~; (2) screening of ~~substances~~a substance which specifically ~~bind to~~binds OBM and ~~inhibit~~inhibits or modifies the biological activity of OBM~~,~~; and (3) screening of ~~proteins~~a protein (OBM receptor) which ~~are present in osteoclast~~exists on a precursor ~~cells~~cell of osteoclasts and trans~~duce~~mits the biological activity of OBM; and (~~OBM receptor~~4). ~~It is also possible to develop~~ as well as developments of antagonists and agonists using this OBM receptor. In ~~the~~ combinatorial chemistry using the above-~~mentioned~~ OBM or OBM receptor, a peptide library ~~used for the screening of the antagonists~~required to identify an antagonist or agonists can be prepared ~~by~~in accordance with the following ~~method. Specifically, one of the~~specific methods. One of them is a split method (Lam *et al.*; Nature 354, 82- to 84, 1991). ~~According to~~In this method, synthetic carriers (beads) ~~each comprising a specific amino acid (unit)~~are bound ~~thereto are prepared~~to amino acids (units), separately ~~for all units~~. ~~The synthesized carriers~~Then, these synthetic beads are mixed ~~altogether~~together and divided into ~~portions~~an equal ~~to the~~ number of ~~the~~ units. ~~Then~~, ~~the next units are~~and then bound. to the subsequent units. ~~This procedure is repeated "~~By repeating this operation n~~"~~ times ~~to produce~~, a library ~~containing carriers to~~in which ~~"~~n~~"~~ units are bound. to the carriers is prepared. ~~According to this synthetic method, each carrier pool has one type of~~Such an operation allows the synthesis of only one sequence. per one group of the carriers. ~~Therefore~~Hence, ~~it is possible to identify a peptide specifically binding to the protein of the present invention by selecting the pool which gives a signal~~ when a positive carrier group is selected in ~~this~~said method for screening ~~method using~~by use of the protein of the present invention~~,~~ and ~~determining~~then the amino acid sequence ~~of the~~thereof is determined, a specifically binding peptide ~~bound on the pool~~can be identified. ~~Another~~As another method, ~~is~~a phage display method ~~which utilizes phage carrying~~can be used. In this method, synthetic ~~DNA which encode~~ genes encoding random peptides ~~with random amino acid sequences~~are expressed using phage.

~~The~~While this method has ~~the~~an advantage ~~of increasing the~~that it can archive a larger number of molecules in ~~the~~a library ~~as compared with~~than the above~~-mentioned~~ synthetic ~~peptide~~ library ~~method~~, ~~but~~it also has ~~the~~a disadvantage that the kind of ~~less variety for a given number of~~peptides per molecules is not as varied because ~~there can be particular~~peptides having sequences ~~which are missing~~that phages don't prefer do not exsist in the library ~~if the phages are unable to express those sequences~~. In the phage display method, as in the case of the split method, using a screening system ~~using~~with the protein of the present invention ~~can also be applied to determine the nucleotide sequence encoding the peptide. That is, the~~ phage specifically binding ~~to the protein of the present invention is~~thereto are concentrated by panning~~, the selected~~. Thus obtained phage ~~is~~are amplified in ~~Escherichia~~*E. coli*, and further, the nucleotide sequence encoding the peptide is determined. ~~In addition~~Furthermore, when it is desired that a specific peptide ~~exhibiting high specificity and~~having high affinity ~~to~~for OBM or OBM receptor ~~can be~~is screened from a peptide library using the screening ~~systems mentioned~~system of the above ~~in~~ (2) ~~and~~or (3), a specific peptide having a very high affinity can be obtained by screening a positive carrier or phage in the co-presence of ~~OBM or~~ OCIF ~~while increasing the~~or OBM with a change of concentration ~~of OBM or OCIF. Only positive carrier pools or phages are selected in this manner~~. For example, screening of a peptide agonist of low molecular weight ~~peptide agonists exhibiting~~having an EPO ~~(erythropoietin)~~-like activity ~~were screened~~ from a varied peptide library ~~using a receptor of~~with an erythropoietin (EPO~~)~~ which is a hematopoietic hormone) receptor, ~~the tertiary~~analysis of a three-dimensional structure thereof, and the production of ~~this~~a substance ~~was analyzed, and based on this tertiary structure,~~(agonist) of low -molecular - weight ~~substances (antagonist) exhibiting the~~having an EPO~~-like~~ activity ~~were synthesized~~through synthesis of organic chemical compounds based on the three-dimensional structure has already been successful (Nicholas *et al.*: Science, 273, 458- to 463, 1996).

~~The present~~Furthermore, the inventors have ~~previously discovered using the osteoclastogenesis inhibitory factor, OCIF,~~found that ~~an OCIF-binding~~a protein binding OCIF is specifically expressed on ~~osteoblastic~~an osteoblast-like stromal cell line, ST2, which was cultured in the presence of ~~a osteotropic~~ bone resorption factors such as the

active-form of vitamin D_3 ~~or~~and parathyroid hormone (PTH), using osteoclastogenesis inhibitory factor (OCIF). ~~The~~Moreover, the inventors ~~further~~have found that ~~this~~e protein ~~exhibits a biological activity to support or stimulate~~, which is associated with differentiation ~~or maturation of osteoclasts from~~ immature osteoclast precursor cells~~, and clarified various~~ to osteoclasts and maturation thereof, has a biological activity as a factor which supports and promotes so-called differentiation and maturation of osteoclasts. After purification of the protein, the physicochemical properties and ~~the~~ biological activity of ~~this protein by purification thereof.~~the protein were examined. ~~In order to compare~~The inventors have compared the physicochemical properties and biological activity of the recombinant protein OBM ~~expressed~~ by expressing the DNA of the present invention ~~and~~ with those ~~above mentioned~~ of a purified natural -type protein which specifically binds ~~to~~ OCIF~~, the present inventors investigated the physicochemical properties and biological activities of the two proteins~~ in order to clarify differences between them. As a result, ~~the two proteins were confirmed ① to be~~they have found that (1) each of both proteins is a membrane-bound proteins ~~which~~and specifically ~~bind to~~binds OCIF~~, ② to have~~; (2) they shared a molecular weights of ~~approximately~~about 40,000 ~~determined~~as measured by SDS-PAGE~~,~~; and ~~③~~(3) ~~to~~they have an apparent molecular weights of about 90,000~ to 110,000 when ~~cross-linked~~crosslinked ~~to~~with a monomer ~~form~~-type OCIF~~. Not only are these~~, which indicates that they have very similar physicochemical properties ~~identical, but both proteins exhibit a biological~~. An activity to support ~~or~~and ~~stimulate~~promote differentiation ~~or~~and maturation of osteoclasts was also shared by them as well. Therefore~~, suggesting~~ the possibility that ~~these~~both proteins are ~~the same protein~~identical was suggested. ~~In addition~~Furthermore, ~~a rabbit~~ an anti-OBM rabbit polyclonal antibody ~~produced using the purified protein~~ prepared ~~by expressing~~with the protein (recombinant OBM), which was genetically expressed with the DNA of the present invention ~~by a genetic engineering technique (recombinant OBM) was confirmed to~~and then purified, has cross ~~react with the above described~~reactivity to the purified natural ~~type protein, to inhibit specific~~-type protein obtained by the above method and specifically inhibited the binding ~~of this purified~~between said natural -type protein and OCIF ~~in the same manner~~, just as ~~the antibody~~it inhibits specific binding ~~of~~between OBM and OCIF. ~~Based on~~From these results, it is ~~clear~~obvious that the

recombinant protein OBM expressed ~~by~~with the DNA of the present ~~invention~~ is identical to the natural -type protein which specifically binds ~~to~~ OCIF.

~~To isolate~~Furthermore, for isolating a gene (cDNA) ~~encoding~~that encodes a human-derived OCIF- binding protein molecule (hereinafter ~~called~~referred to as "human OBM") which specifically binds ~~to~~ OCIF and ~~exhibits~~has ~~the~~an activity to support and ~~stimulate~~promote differentiation ~~and maturation of osteoclasts from~~ mouse spleen cells ~~in the same manner~~to osteoclasts and maturation, just as the natural -type or the recombinant mouse OBM ~~dose, a cDNA library prepared from mRNA derived from human lymph nodes was screened using a human OBM cDNA fragment as a probe. The human OBM cDNA fragment was obtained by~~does, the inventors have carried out a polymerase chain reaction (PCR) ~~in accordance with the method mentioned~~using primers prepared based on the above ~~using both~~mouse OBM cDNA ~~prepared from~~and human lymph node-derived cDNA as a template ~~and the primer which was prepared from mouse~~. Thus, the inventors have screened said cDNA library with the obtained human OBM ~~cDNA~~cDNA fragment. As a result, they have succeeded in isolation of the cDNA ~~encoding~~which encodes the human-derived protein which specifically binds ~~to~~ OCIF ~~was~~(human ~~isolated~~OBM) and determination of the nucleotide sequence of ~~the~~said cDNA ~~encoding this human OCIF-binding protein molecule (i.e. the cDNA encoding human OBM) was determined~~. ~~Similar to mouse OBM, this~~They have found that human OBM encoded by the cDNA ~~has characteristics to bind to OCIF~~ strongly and specifically binds OCIF on ~~the~~a cell membrane and ~~exhibits the~~has a biological activity to support and promote differentiation ~~and maturation of osteoclasts from~~ mouse spleen cells to osteoclasts and maturation thereof, just as mouse OBM does. ~~Specifically~~That is, other objects of the present invention ~~provides~~ are to provide: (1) DNA ~~encoding~~which encodes human OBM which is a novel human ~~OBM~~-derived protein which binds ~~to~~ osteoclastogenesis inhibitory factor (OCIF~~),~~; a protein ~~which possesses the~~having an amino acid sequence encoded by the DNA~~,~~) (2) a method for genetically producing ~~the~~a protein ~~exhibiting characteristics of~~which specifically ~~binding to~~binds OCIF and ~~the~~has an activity to support and promote differentiation ~~and maturation of osteoclasts from~~ mouse spleen cells to osteoclasts and maturation thereof by ~~genetic engineering techniques, pharmaceutical compositions comprising this protein~~use of the DNA; (3) an

agent for ~~the treatment of diseases caused by abnormality of~~treating bone metabolism~~,~~ abnormality comprising the protein; (4) a method for screening ~~substances regulating~~a substance which controls expression of human OBM~~,~~; (5) a method for screening ~~substances~~a substance which ~~inhibit~~binds human OBM and inhibits or ~~modulate the activity of human OBM by binding to it,~~ modifies an effect thereof; (6) a method for screening ~~receptors~~a receptor which ~~bind to~~binds human OBM and ~~transmit the action of OBM,~~transmits an effect thereof; and (7) a pharmaceutical compositions comprising ~~the~~a substances obtained ~~by~~as a result of these ~~screenings~~methods for screening.

The present invention ~~further provides~~relates to DNA ~~encoding~~which encodes human OBM, a novel human ~~OBM~~ protein, which specifically binds ~~to~~ OCIF and ~~exhibits~~has ~~the~~a biological activity to support and promote differentiation and maturation of osteoclasts~~,~~; a protein ~~which possesses the~~having an amino acid sequence encoded by the DNA~~,~~; a method for genetically producing ~~the~~a protein ~~exhibiting characteristics of~~which specifically ~~binding to~~binds OCIF and ~~the~~has an activity to support and promote differentiation and maturation of osteoclasts ~~by genetic engineering techniques,~~with the DNA; and ~~pharmaceutical compositions comprising this protein~~an agent for ~~the treatment of diseases causing abnormality of~~treating bone metabolism abnormality comprising the protein. Furthermore, the present invention ~~provides~~also relates to a method for screening ~~substances regulating~~a substance which controls expression of human OBM~~,~~; a method for screening ~~substances~~a substance which ~~inhibit~~binds human OBM and inhibits or ~~modulate the activity of human OBM by binding to it, a~~modifies an effect thereof; a method for screening ~~receptors binding to human OBM and transmitting the action of OBM, antibodies against~~a receptor which binds human ~~OCIF binding protein,~~ OBM and transmits a biological activity of OBM; a pharmaceutical compositions comprising a substance obtained as a result of these ~~antibodies~~methods for screening; an antibody to the ~~prevention~~ human-derived OCIF binding protein; and an agent for preventing and/or ~~treatment of diseases causing abnormality of~~treating bone metabolism abnormality using the antibody.

The novel~~,~~ human-derived OCIF- binding protein molecule ~~(~~, human OBM~~)~~ ~~which is,~~ encoded by the DNA of the present invention ~~has~~shows the following physicochemical properties and biological activity. That is, (a) ~~binds~~ human OBM

specifically binds to osteoclastogenesis inhibitory factor (OCIF) (WO 96/26217)~~,~~; (b) ~~has~~ human OBM shows a molecular weight of ~~approximately~~about 40,000 (~~+~~± 5,000) ~~when~~as ~~determined~~measured by SDS-PAGE under reducing conditions and shows an apparent molecular weight of ~~approximately~~about 90,000~~-~~ to 110,000 when crosslinked with a monomer ~~form~~-type OCIF~~,~~; and (c) ~~exhibits~~ human OBM has a biological activity to support and ~~stimulate~~promote differentiation and maturation of osteoclasts.

~~Mouse OBM~~The cDNA ~~which encodes~~encoding mouse OBM, mouse-derived OCIF- binding protein ~~and used~~, useful as a probe ~~to~~for ~~isolate~~separating and identifying the cDNA ~~encoding~~which encodes human OBM of the present invention, can be isolated ~~according to the above-mentioned method~~ from a cDNA library of a mouse ~~osteoblastic~~osteoblast-like stromal cell line, ST2. ~~Human~~Furthermore, human osteoclastogenesis inhibitory factor (OCIF) ~~which is necessary~~, required to ~~evaluate~~examine the properties and ~~the~~ biological activity of the protein obtained by ~~expression of~~expressing human OBM cDNA, can be ~~prepared according to the method described in WO 96/26217 by isolating~~isolated from ~~a~~the culture ~~broth~~solution of human fibroblast ~~cell line, IMP~~strain IMR-~~90,~~90 in accordance with the method described in WO 96/26217, or ~~by genetic engineering techniques using~~it can be genetically produced with the DNA encoding ~~OCIF~~it. ~~Recombinant human OCIF~~To examine the properties and biological activity of human OBM, recombinant humOCIF, recombinant mouse OCIF, recombinant rat OCIF~~,~~ ~~or~~and the like can also be used ~~for the assessment of the properties and biological activity of human OBM~~. These recombinant OCIFs can be obtained ~~according to conventional methods~~ by ~~inserting cDNA~~incorporating the corresponding cDNAs into ~~an~~ expression ~~vector~~vectors in accordance with a commonly used method, expressing ~~the cDNA~~OCIFs in animal or insect cells such as CHO cells, BHK cells~~,~~ ~~or~~and Namalwa cells, ~~or in insect cells,~~ and purifying ~~the expressed proteins~~them.

~~The following methods can be used to isolate~~Methods for isolating the human cDNA ~~encoding~~which encodes the target protein (cDNA cloning)~~.~~ include: ~~①~~(1) ~~A~~a method comprising the steps of purifying the protein, determining ~~the~~a partial amino acid sequence ~~of the protein~~thereof, and isolating the target cDNA by a hybridization ~~using the~~with DNA ~~fragment~~having ~~comprising~~a nucleotide sequence corresponding to the

amino acid sequence as a probe, ② (2) a method ~~applied even in the case where the amino acid sequence~~(expression cloning method) comprising the steps of ~~the protein is not known, which comprises~~ constructing a cDNA library ~~in~~with ~~a~~an expression vector, regardless of whether the amino acid sequence of the target protein is unknown, introducing ~~the cDNA library~~them into cells, and screening for the presence and absence of the expression of the target protein so as to isolate the ~~objective~~target cDNA ~~(expression cloning method),~~; and ③ (3) a method of isolating the cDNA ~~encoding~~which encodes the target human protein ~~from~~by the hybridization or polymerase chain reaction (PCR) method from cDNA library constructed ~~using~~from human cells or ~~tissues by hybridization~~tissue or ~~by the use of polymerase chain reaction (PCR) using the~~ cDNA ~~encoding the~~which encodes a protein ~~of mammalian origin (~~derived from a mammal other than human~~) which possesses~~ and having the same ~~characteristics~~properties and biological activity ~~as the~~of the human-derived target protein ~~of human origin~~ as a probe~~,~~ ~~assuming that the cDNA probe has~~, based on an assumption that the cDNA which encodes the non-human protein shares high homology with ~~the human origin cDNA which~~ that which encodes the desired corresponding human protein to be cloned.

Based on ~~the assumption that human OBM cDNA has a high homology with~~an assumption that human OBM cDNA is highly homalogous with the above mouse OBM cDNA, ~~it is possible to determine which~~human cells or tissues produce~~ing~~ human OBM can be identified by Northern hybridization method using the latter (mouse ~~OBM~~) cDNA as a probe. Human OBM cDNA can be cloned as follows. A human OBM cDNA fragment is obtained ~~by the following method using the~~ through PCR using mouse OBM ~~primer prepared from the~~ primers prepared based on the mouse OBM cDNA and the cDNA. ~~Human OBM cDNA fragments can be prepared by the PCR method using cDNA prepared from~~ library of a cell or tissue which produces human OBM ~~producing tissues such as~~ (e.g., a human lymph ~~nodes~~node) as identified above, as primers and a template, respectively. ~~These human OBM cDNA fragments are used as probes for screening the~~The cDNA library of ~~human OBM producing~~ cells or tissues which ~~were~~produce human OBM as identified ~~according to the method mentioned~~ above is screened with the human OBM cDNA fragment as a probe, and thus, human OBM cDNA can be obtained. The present invention relates to the ~~DNA encoding human OBM which has~~

~~characteristics of specific binding to~~obtained DNA that encodes human OBM, a human-derived protein which specifically binds OCIF and ~~exhibits~~has biological activity to support and promote differentiation and maturation of osteoclasts. ~~Because~~Since ~~the~~human OBM ~~which is~~ encoded by the DNA of the present invention is a me~~a~~mbrane-bound ~~type~~ protein ~~which comprises~~having a transmembrane domain, ~~this protein~~it can be detected by labeling OCIF and ~~by examining the~~then binding ~~of~~ the labeled OCIF to the surface of an animal cells in which the cDNA of the present invention ~~was~~is expressed. ~~The above-described~~In such a case, OCIF can be labeled by a method which is conventinally used for labeling ~~method using~~ protein such as labeling with a radioisotope ~~or fluoresceine conventionally applied to~~and fluorescence labeling ~~proteins can be used for labeling OCIF~~.

The molecular weight of the protein expressed by ~~the~~ human OBM cDNA of the present invention ~~can be accessed~~is determined by gel filtration chromatography, SDS-PAGE~~,~~ ~~or~~and the like. ~~In order to accurately~~To determine the molecular weight more accurately, ~~it is desirable to use the~~ SDS-PAGE ~~method~~is preferably used, ~~by which~~and human OBM ~~was~~is ~~specified~~identified as a protein having a molecular weight of ~~approximately~~about 40,000 (40,000 ~~+~~± 5,000) under reducing conditions.

~~Comparatively~~An example of DNA hybridization under relatively mild conditions ~~for hybridization of DNA~~ in the present invention ~~are the conditions, for example, wherein~~is that after DNA is transferred to a nylon membrane and ~~immobilized thereto~~ fixed in accordiangce ~~to~~ with a ~~conventional~~commonly used method ~~and~~, the DNA is hybridized with another radiolabeled DNA as a probe ~~DNA labeled with an isotope~~ in a ~~buffer solution for~~ hybridization buffer at ~~a temperature of~~ 40 ~~°~~ to 70°C for about 2 hours to overnight~~, followed by washing in~~ and then washed with 0.5 ~~×~~X SSC (0.075 M sodium chloride and 0.0075 M sodium citrate) at 45°C for 10 minutes. ~~Specifically~~More specifically, ~~High~~bondafter DNA is transferred and fixed to a nylon membrane, which is HYBOND® N (Amersham Co., Ltd.) ~~is used as~~, in accordance with a conventional method, the ~~nylon membrane to transfer and immobilize DNA thereon. The DNA is then~~DNA is hybridized with another ^{32}P-labeled DNA as a probe ~~DNA labeled with ^{32}P~~ in ~~a rapid hybridization buffer~~Rapid Hybridization Buffer (Amersham Co., Ltd.) at 65°C

for 2 hours, ~~followed by~~ and then washinged with the above 0.5~~*~~X SSC at 45°C for 10 minutes.

~~A~~As a representative *in vitro* culture system for osteoclastogenesis, a co-culture system of mouse-derived ~~osteoblastic~~osteoblast-like stromal ~~cells~~cell line, ST2, and mouse spleen cells in the presence of the active-form of vitamin D_3 or PTH is well known ~~as a typical in vitro culture system for osteoclast formation. Interaction by adhesion of osteoblastic~~ For promoting osteoclastogenesis in this *in vitro* culture system, both the interaction between a osteoblast-like stromal cells and a spleen ~~cells~~cell through their binding, and the presence of ~~an osteotropic~~ bone resorption factors such as the active-form of vitamin D_3 ~~or~~and PTH are ~~indispensable for the osteoclasts formation in this in vitro culture system~~essential. In this *in vitro* culture system, a recombinant COS ~~cells, monkey kidney cells having no osteoclast formation supporting capability, acquire capability to support osteoclasts formation~~cell strain, resulting from ~~spleen cells in the absence of an osteotropic factor when~~the expression of the cDNA of the present invention ~~was expressed as osteoblastic~~thereon, has obtained an ability to support osteoclast formation from spleen cells, just like the osteoblast-like stromal cell line ST~~2 did~~2, while COS-7 cells (a monkey kidney-derived cell line) does not have an ability to support osteoclast formation in the absence of said bone resorption factors. ~~Based on the fact that~~Furthermore, since the cDNA of the present invention encodes a membrane-bound protein ~~comprising a transmembrane domain form~~, this~~e cDNA~~protein can be expressed as a secreti~~onry form~~-type or solubilized ~~form by~~type protein after removing the ~~part~~fragment which encodes ~~this transmembrane~~the membrane binding domain thereof. It ~~was~~has also been confirmed that osteoclastogenesis ~~can be formed~~ was promoted simply by adding the ~~addition of the secretion form~~secretory-type human OBM to the above ~~mentioned~~ *in vitro* culture system in the absence of ~~osteotropic~~said bone resorption factors. ~~Based on~~From these results, ~~the~~ human OBM ~~which is~~ encoded by the cDNA of the present invention is ~~specified~~identified as ~~the~~a factor involved in ~~the~~ differentiation and maturation of osteoclasts.

~~A recombinant~~Recombinant human OBM can be pre~~par~~oduced by inserting the cDNA of the present invention into an expression vector~~, preparing~~ so as to prepare a plasmid for expressing human OBM ~~expression plasmid,~~ and then introducing and

expressing the plasmid ~~into~~in various ~~cell~~cells and strains ~~and expressing OBM in the cells. Mammalianian cells such as~~ COS-7, CHO~~,~~ and Namalwa cells~~, or bacteria such~~ and the like can be used as ~~Escherichia~~mammalian host cells suitable for expression, and *E. coli* and the like can be used as ~~a~~bacterial host ~~for expressing OBM~~cells. In thiose cases, recombinant human OBM ~~may~~can be expressed as a membrane-bound ~~form~~ protein~~,~~ by using the full length of DNA~~,~~ or as a secreti~~on~~ry ~~form~~type or solubilized ~~form~~type protein by removing a region which encodes the ~~part encoding the transmembrane~~membrane binding domain. ~~The~~Thus produced recombinant human OBM ~~thus produced~~ can be purified efficiently ~~purified using a suitable~~in combination ~~of~~ wiht conventionally ~~purification~~ used methods ~~used~~ for ~~common~~purifying proteins such as affinity chromatography using OCIF ~~-~~immobilized ~~columns~~or a column, ion exchange chromatography, ~~and~~ gel filtration chromatography and the like. ~~Human~~Thus obtained human OBM of the present invention ~~thus obtained~~ is useful, due to its activity, as a medicament, e.g., as an agent for treating ~~diseases caused by abnormality of~~ bone metabolism abnormality such as osteopetrosis or as ~~a~~an experimental and diagnostic reagent ~~for research and diagnosis of such diseases~~.

The ~~following screening operations can be carried out using the~~human OBM protein ~~OBM~~ encoded by the ~~DNA~~cDNA of the present invention enables: (1) screening of ~~substances~~a substance which ~~can regulate~~controls expression of human OBM~~,~~; (2) screening of ~~substances~~a substance which specifically ~~bind to~~binds human OBM and inhibits or modif~~y~~ies the biological activity of human OBM~~,~~; and (3) screening of a human ~~proteins~~protein (human OBM receptor) which ~~are present~~exists in ~~osteoclast~~a precursor ~~cells~~cell of human osteoclasts and transmits the biological activity of human OBM ~~(human OBM receptor). It is also possible to develop antagonists~~, as well as development of antagonist and agonists using this human OBM receptor. In ~~the~~ combinatorial chemistry using the above human OBM or human OBM receptor, a peptide ~~libraries required~~library, which is employed for ~~the screening~~identification of ~~antagonists~~an antagonist or ~~agonists~~agonist, can be ~~produced by~~prepared in accordance with the same method ~~as used for the screening~~ using the mouse OBM. ~~A~~After screening the peptide ~~with extremely~~ library by said method in which human OBM is used instead

of mouse OBM, a specific peptide having very high ~~specificity and~~ affinity can be obtained ~~by screening peptide libraries using human OBM instead of mouse OBM~~.

~~Although this OBM is very~~Furthermore, for measurement of OBM, a highly useful ~~as~~protein ~~mentioned~~described above ~~and antibodies~~, it is necessary to obtain an antibody which specifically recogniz~~inges~~ OBM and establish an enzyme immunoassay using ~~these antibodies are indispensable in determination of OBM concentration~~it. However, no antibod~~ies~~y useful for ~~the access~~measurement of OBM ~~concentration have~~has been ~~so far~~ available. ~~In addition~~Moreover, an anti-OBM ~~antibody or anti-~~/sOBM antibody which neutralizes the biological activity of OBM or sOBM is ~~supposed~~assumed to suppress ~~the~~an activity of OBM or sOBM~~, specifically the activity~~ to ~~induce~~promote osteoclast~~s~~ formation~~. These,~~ ~~are~~and expected to be ~~useful~~developed as ~~therapeutic agents to treat abnormality of~~an agent for treating bone metabolism abnormality. However, ~~no~~ such ~~antibodies have so far~~an antibody has not been available.

~~In view of this situation~~Under that circumstance, the ~~present~~ inventors have ~~conducted extensive~~made intensive studies~~. As a result, the present inventors~~ and have found antibodies (anti-OBM/sOBM antibodies) which recognize both ~~OBM~~of the following antigens, a membrane-bound protein (OCIF binding molecule; OBM) which specifically binds ~~to~~ osteoclastogenesis inhibitory factor (OCIF)~~,~~ and a soluble-type OBM (sOBM) ~~which lack a transmembrane~~lacking the membrane binding domain. ~~Accordingly~~Therefore, objects of the present invention ~~provides antibodies~~are to provide: (1) an antibody (anti-OBM/sOBM antibod~~ies~~y) which recognizes both ~~OBM~~of the following antigens, a membrane-bound protein (OBM) which specifically binds to osteoclastogenesis inhibitory factor (OCIF)~~,~~ and soluble OBM (sOBM ~~which lack a transmembrane~~) lacking the membrane binding domain; (2) a method for ~~the preparation~~production thereof; (3) a method for ~~determination of~~measuring OBM and the sOBM ~~concentrations using these antibodies~~by use of said antibody; and ~~agents~~(4) an agent for ~~the prevention~~ preventing and/or ~~treatment of diseases resulting from abnormality of~~treating bone metabolism abnormality which comprises said antibody as an active ingredient.

The present invention relates to ~~antibodies~~: (1) an antibody (anti-OBM/sOBM antibod~~ies~~y) which recognize~~s~~ both of the ~~OBM~~following antigens, a membrane-bound

protein (OCIF binding molecule; OBM) which specifically binds ~~to~~ osteoclastogenesis inhibitory factor (OCIF)~~,~~ and a soluble-type OBM (sOBM) ~~which lack a transmembrane~~lacking the membrane binding domain; (2) a method for ~~the preparation~~production thereof; (3) a method for ~~quantifying~~measuring OBM and the sOBM ~~using these antibodies~~by use of said antibody; and ~~agents~~(4) a pharmaceutical compisition comprising said antibody as an active ingredient, particularly, an agent for ~~the prevention~~ preventing and/or ~~treatment of diseases resulting from abnormality of~~treating bone metabolism abnormality. ~~The antibodies~~

An antibody of the present invention ~~exhibit~~is an antibody which has an activity ~~of neutralizing the~~to neutralize osteoclastogenesis ~~accelerating~~promoting activity, which is ~~the~~a biological activity that OBM and sOBM have, said antibody has any of ~~OBM and sOBM and comprises the antibodies having~~ the following ~~characteristics~~properties:~~(~~ a) a polyclonal antibody which recognizes both mouse OBM and mouse sOBM antigens (anti-mouse OBM/sOBM polyclonal antibody)~~,(~~; b) a polyclonal antibody which recognizes both human OBM and human sOBM antigens (anti-human OBM/sOBM polyclonal antibody)~~,(~~; c) a monoclonal antibod~~ies~~y which recognizes both mouse OBM and mouse sOBM antigens (anti-mouse OBM/sOBM ~~monoclonal~~polyclonal antibod~~ies~~y)~~,(~~; d) a monoclonal antibod~~ies~~y which recognizes both human OBM and human sOBM antigens (anti-human OBM/sOBM ~~monoclonal~~polyclonal antibod~~ies~~y)~~,~~; and~~(~~ e) an anti-human OBM/sOBM monoclonal antibod~~ies~~y which ~~crossreact~~has crossreactivity to both mouse OBM and mouse sOBM antigens.

The polyclonal antibody which recognizes both mouse OBM and mouse sOBM antigens (hereinafter ~~called~~ referred to as "anti-mouse OBM/sOBM polyclonal antibody") and the polyclonal antibody which recognizes both human OBM and human sOBM antigens (hereinafter ~~called~~ referred to as "anti-human OBM/sOBM polyclonal antibody") ~~were produced~~can be obtained by the following ~~method~~means. ~~The~~A purified mouse OBM ~~used~~ as an antigen for immunization can be obtained ~~according to~~in accordance with the above-~~mentioned~~ method. ~~Especially~~That is, natural-type mouse ~~osteoblastic~~OBM can be obtained by treating a mouse osteoblast-like stromal cell line, ST2, ~~was treated~~ with the active-form of vitamin D_3~~,~~ and ~~OBM on~~subsequently purifying it from the cell ~~membrane was purified using an~~membranes of said cell by

means of OCIF- immobilized on a column and gel filtration chromatography~~, thereby obtaining natural mouse OBM (native OBM)~~. ~~The~~Alternatively, after incorporating the above~~-mentioned~~ mouse OBM cDNA (~~Sequence Table, Sequence No.~~SEQ ID NO: 15) or human OBM cDNA (~~Sequence Table, Sequence~~SEQ ID ~~No.~~NO: 12) ~~was inserted~~ into an expression vector~~ according to conventional methods. Recombinant mouse OBM (Sequence Table, Sequence ID No. 1) and recombinant human OBM (Sequence Table, Sequence ID No. 11) can be obtained by~~ expressing ~~cDNA~~OBM in an animal ~~cells~~or insect cell such as a CHO cells~~,~~ a BHK cells, Namalwa~~,~~ or a COS-7 ~~cells, insect cells~~cell or ~~Escherichia~~ *E. coli*~~,~~ and then purifying ~~them using~~by the same ~~purification methods~~method as ~~mentioned~~described above~~. These~~, recombinant mouse OBM (SEQ ID NO: 1) or recombinant human OBM (SEQ ID NO: 11) can be obtained, and these may also be used as antigens for immunization. ~~In~~At this ~~instance~~time, ~~purifying~~it takes tremendous effort to highly purify a large ~~amount and a high level~~quantity of mouse OBM or human OBM~~ which are~~, a membrane-bound ~~proteins is a task requiring a great deal of labor~~protein (OBM). On the other hand, ~~as mentioned above, OBM which~~it has been confirmed that there is no difference in ability to promote differentiation and maturation of osteoclasts between OBM, a membrane-bound protein, and ~~a~~ soluble-type OBM (sOBM), which is a soluable protein obtained by deleting ~~transmembrane~~the membrane binding domain of OBM ~~are known to be almost the same in their osteoclast differentiation and maturation activities~~as described above. ~~It is possible to use~~Accordingly, taking into account that expression and high purification of mouse sOBM and human sOBM~~ which~~ are relatively ~~easily expressed and purified to a high level~~easy, these sOBMs, solubilized proteins, may be used as antigens for immunization. Mouse sOBM (~~Sequence Table, Sequence~~SEQ ID ~~No.~~NO: 16) and human sOBM (~~Sequence Table, Sequence~~SEQ ID ~~No.~~NO: 17) can be obtained by adding a nucleotide sequence~~ encoding~~, which encodes a known signal sequence ~~originating~~derived from ~~the~~ other ~~secretion protein in the~~secretory-type proteins, 5' upstream ~~side~~ of the ~~5' end of, respectively,~~ mouse sOBM cDNA (~~Sequence Table, Sequence~~SEQ ID ~~No.~~NO: 18) ~~and~~or the human sOBM cDNA (~~Sequence Table, Sequence~~SEQ ID ~~No.~~NO: 19), ~~inserting these~~incorporating the cDNA into an expression vector ~~by the use of genetic~~in accordance with the same gene engineering ~~techniques, causing these proteins to be~~

~~expressed in host cells such as various~~method as described above, expressing the protein in a variety of animal cells, insect cells~~,~~ or ~~Escherichia~~*E. coli* as a host, and then purifying ~~the resultant products~~. ~~The antigens~~Thus obtained antigen for immunization ~~thus obtained are~~is dissolved in a phosphate buffered saline solution (PBS) and, if necessary, mixed with ~~the~~an ~~same~~equal volume of Freund's complete adjuvant ~~to emulsify~~and emulsified. Then, an animal is immunized with the ~~solution if required, and subcutaneously administered to animals about once~~emulsion through a ~~week to immunize these animals several~~few times of subcutaneous administration with a one-week interval between each. ~~A booster injection is given when the~~The antibody titer is measured. When the value reaches ~~a~~ maximum~~. Exsanguination~~, booster administration is performed. On the 10~~days after~~th day from the booster administration, all the blood was collected. The ~~resulting~~obtained antiserum is ~~treated~~fractionated and precipitated with ammonium sulfate ~~precipitation. IgG~~, and the globulin fraction is purified ~~using~~with an anion exchange chromatography or the antiserum is diluted twice with Binding Buffer (Bio-Rad Co., Ltd.) and the diluted antiserum is purified by ~~p~~Protein A- or ~~p~~Protein G-~~Sepharose~~ SEPHAROSE® (Pharmacia Co., Ltd.) column chromatography ~~after diluting the antiserum two-fold with Binding Buffer™ (BioRad Co.)~~ Thereby, ~~to obtain~~ the desired anti-mouse or anti-human OBM/sOBM polyclonal antibody can be obtained.

The monoclonal antibod~~ies~~y of the present invention can be obtained ~~according to~~by the following method. ~~In the same manner~~That is, as ~~in the case of the polyclonal antibodies,~~an antigen for immunization required to prepare the monoclonal antibody, a natural-type mouse OBM ~~(native OBM),~~, recombinant mouse or human OBM, or recombinant mouse or human sOBM can be used, as ~~immunogens to prepare monoclonal antibodies~~ used in preparation of the above polyclonal antibody. ~~Hybridomas are produced according to conventional methods by immunizing~~Lymphocytes derived from immunized mammals with ~~these~~each antigens or that obtained by ~~immunizing lymphocytes~~ *in vitro* ~~and fusing the immunized cells~~methods are fused with a myeloma ~~cells~~cell line, and hybridomas are prepared in accordance with a conventional method. ~~By analyzing~~From the ~~hybridoma~~ culture ~~supernatant thus obtained~~of this hybridoma, a hybridoma producing an antibody which recognizes each antigen is selected by ~~a~~ solid-phase ELISA ~~method, antibody-producing hybridomas recognizing the~~, using each highly

purified antigen ~~are selected~~. The ~~resulting~~ obtained hybridomas ~~are~~ is cloned, and ~~established as~~a thus obtained stable antibody-producing hybridoma ~~clones. These hybridomas are~~is cultured ~~to obtain~~. The target antibody can be obtained therefrom. For preparation of the ~~antibodies. Small mammals~~hybridoma, immunizing a mammal, a small animal such as ~~mice~~a mouse or ~~rats~~rat, ~~are~~is commonly used ~~to produce hybridomas~~. ~~Animals are immunized by intravenously or intraperitoneally injecting the antigen diluted to a suitable concentration using a suitable~~To immunize the animal, a method comprising the following steps is conventinally used: diluting the antigen with an appropriate solvent such as physiological salt~~ine~~ solution. ~~Optionally~~ to an appropriate concentration and then administering the solution and, if necessary, co-administering Freund's complete adjuvant ~~maybe used together with antigen~~, into vein (i. ~~These are usually injected~~v.) or the abdominal cavity (i.p.), about 3- or 4 times~~, once~~ in total with a 1 to 2-week ~~or every two weeks~~interval between each. The immunized animals ~~are~~is dissected ~~three days~~on the 3rd day after ~~final~~the last immunization. ~~Splenocytes~~, and spleen cells are obtained from the ~~removed~~ isolated spleen ~~are~~ and used as immunocytes (immunized cells). ~~As~~Illustrative examples of mouse-derived myeloma ~~to be~~ for cell fuse~~d~~ion with ~~immunized cells~~,the immunocytes include p3/x63-Ag8, p3-U1, NS-1, MPC-11, SP-2/0, ~~FO~~,F0, P3x63 Ag~~8.653~~,8. 653 and S~~194 can be given.~~194. ~~A~~Furthermore, illustrative examples of rat-derived cells include cell lines such as R-~~210 is given as the cell of rat origin.~~210. ~~Human antibodies are produced by immunizing~~For producing human antibody, human B ~~lymphocytes~~lymphocyte cells are immunized *in vitro* and ~~fusing the immunized cells~~fused with human myeloma cells or a cell line transformed ~~with~~by EB virus. ~~The fusion~~Fusion of ~~the~~an immunized ~~cells and~~cell with a myeloma ~~cells can be carried out~~cell line is performed according to a ~~conventional~~known method~~s~~ such as ~~the method~~that of Koehler and Milstein *et al.*(Koehler *et* ~~a1.~~*al.*: Nature 256, 495-~~497~~ (to 497, 1975)~~). A~~, while an electric pulse method using an electric pulse ~~is~~may also ~~applicable~~be used. Immunized ~~lymphocytes~~lymphocyte cells and myeloma ~~cells~~cell lines are mixed together at a ratio conventionally ~~accepted ratio~~ used and fused in ~~an FCS-free (fetal~~common bovine fetal serum (FCS)-free~~)~~ medium for cell culture ~~medium with an addition of~~in which polyethylene glycol is added. Then, ~~and~~ ~~cultured~~culture is carried out in ~~an~~ FCS-containing HAT selecti~~on~~ve medium so as to

select a fused cells (hybridomas). ~~Next~~Then, ~~the~~ a hybridomas ~~which produce~~ producing an antibod~~iesy were~~ is selected by ~~using~~ a ~~conventional antibody detection~~commonly used method for detecting antibody such as ~~an~~ ELISA, ~~a~~ plaque ~~technique, Ouchterlony~~ method, ouchterlony method or ~~aggregation~~condensation method. Thereafter, ~~to establish stable hybridomas~~a hybridoma is established. The ~~hybridomas~~ established ~~in this way~~hybridoma can be subcultured by a ~~conventional~~common method for culture ~~method or~~and can be stored ~~by freezing as required~~in a frozen state if necessary. ~~A~~The hybridoma ~~can~~may be cultured ~~by~~in accordance with a ~~conventional~~conventionally used method ~~to collect the culture supernatant~~ or ~~implanted~~transplanted in the abdominal cavity of ~~mammals to obtain~~ the mammal. The antibody can be collected from the ~~ascitic fluid~~resulting culture solution or ascites, respectively. The antibody in the culture ~~supernatant~~solution or ~~ascitic fluid~~ascites can be purified by a ~~conventional~~commonly used method such as a salting -out method, ion exchange ~~and~~chromatography, gel filtration chromatography, or ~~p~~Protein A or ~~p~~Protein G affinity chromatography. Almost all the monoclonal antibodies obtained by the above-described method using sOBM as an antigen are antibodies which can specifically recognize not only sOBM but also OBM (~~such antibodies are~~ hereinafter ~~called~~ referred to as "anti-OBM/sOBM monoclonal ~~antibodies~~antibody"). These antibodies can be used for ~~the quantification of OBM or sOBM. The amounts~~measurements of OBM and sOBM ~~can be quantified by labeling~~. After these antibodies are labeled with a radioisotope or an enzyme and ~~by applying the labeled antibodies~~thus employed to ~~a quantification system~~measurement systems known such as ~~a~~as radioimmunoassay (RIA) ~~or enzymeimmunoassay~~and enzyme immunoassay (EIA), an amount of OBM and sOBM can be measured thereby. ~~Using~~By use of these ~~quantification~~measurement systems, ~~the~~an amount of sOBM in a ~~biological~~living sample such as blood or urine can be ~~determined~~measured with ease ~~at~~and with high sensitivity. ~~In addition, the~~Furthermore, by use of these antibodies, an amount of OBM ~~binding~~bound to the surface of a tissue or ~~surface of cells~~cell can be measured through a binding assay or the like with ease ~~at~~and with high sensitivity ~~utilizing a binding assay using these antibodies~~.

When ~~an~~the obtained antibody is used as a medicament~~io~~ for humans, it is desirable ~~to use~~, in consideration of a problem of antigenicity, that a human-type anti-

human OBM/sOBM antibody ~~in view of antigenicity~~ is prepared. The human-type anti-human OBM/sOBM antibody can be prepared ~~according to~~by the following methods ~~①, ②, or ③~~. ~~In the method ①~~That is, (1) human ~~lymphocytes collected~~lymphocyte cells extracted from human peripheral blood or the spleen are ~~immunized~~sensitized *in vitro* with ~~an antigen~~ human OBM ~~or~~ or human sOBM ~~in vitro~~ as an antigen in the presence of IL-~~4. The resulting immunized~~4, and then the sensitized human ~~lymphocytes~~lymphocyte cells are fused with K_6H_6/B_5 (ATCC CRL1823), which is a hetero -hybridoma of mouse and human, ~~and~~thereby, a hybridoma producing the desired antibody is screened ~~to obtain the objective~~. An antibody ~~producing hybridoma. The antibodies~~ produced ~~by the resulting antibody producing hybridomas are~~ from the obtained hybridoma is a human -type anti-human OBM/sOBM monoclonal antibod~~ies~~y. ~~The antibodies neutralizing~~Among these antibodies, an antibody which neutralizes the activity of human OBM/sOBM ~~are~~is selected ~~from these antibodies~~. However, ~~in general,~~ it is usually difficult to ~~produce~~obtain an antibody ~~exhibiting~~ having high affinity ~~to~~ for an antigen ~~by the~~through such a method of ~~immunizing~~sensitizing human ~~lymphocytes~~lymphocyte cells *in vitro*. Therefore, ~~in order to obtain~~for obtaining a monoclonal antibod~~ies~~y ~~with~~ having high affinity ~~to~~for human OBM and sOBM, it is necessary to ~~increase the affinity of the human type~~modify an anti-human OBM/sOBM monoclonal ~~antibodies obtained by the~~antibody with low affinity as described above ~~method. This can~~to be ~~done according to the following method~~that with high affinity. ~~First, a~~A random mutation is introduced into a CDR region (~~particularly~~ CDR-3 ~~region~~in particular) of ~~a~~said human-type anti-human OBM/sOBM monoclonal antibody ~~which neutralize OBM but have a~~with low affinity~~, and make the~~ which a neutralizing antibody obtained as described above. This is expressed with phage ~~to express protein~~. Phages which ~~can~~ strongly bind ~~to~~ human OBM/sOBM ~~which~~as the antigen are selected by ~~a~~ phage display method using ~~plates on~~a plate in which human OBM/sOBM ~~antigens are~~is immobilized. The ~~selected phages are grown~~phage is allowed to proliferate in ~~Escherichia~~*E. coli*. ~~The~~, and the deduced amino acid sequence of the CDR ~~which exhibits~~having high affinity is determined ~~from~~based on the nucleotide sequence ~~of the DNA cloned in the phage~~thereof. The ~~thus~~-obtained ~~DNA encoding~~gene which encodes the human -type anti-human OBM/sOBM monoclonal antibod~~ies~~y is ~~introduced into~~incorporated and expressed in a

~~commonly~~conventionally used expression vector for mammalian cells ~~to produce~~, and then human-type anti-human OBM/sOBM monoclonal antibodies can be obtained. Among them, the desired human -type anti-human OBM/sOBM monoclonal ~~antibodies. The target human type anti-human OBM/sOBM monoclonal antibodies exhibiting high affinity and capable of neutralizing~~antibody which neutralizes the biological activity of human OBM/sOBM and has high affinity thereto can be selected ~~from these monoclonal antibodies. In the method~~ ②Furthermore, ~~mouse type anti-human OBM/sOBM monoclonal antibodies are produced according to the same method as in the present invention~~(2) using ~~BALB~~a Balb/c mouse, an anti-human OBM/sOBM mouse monoclonal antibody is prepared according to a conventinally used method (Koehler *et al.* ~~:~~, Nature 256, 495-~~49,~~ to 497, 1975) as in the present invention, and a monoclonal antibod~~iesy~~ which ~~can neutralize~~neutralizes the biological activity of human OBM/sOBM and ~~exhibiting~~has high affinity ~~are~~thereto is selected. ~~These high affinity mouse anti-human OBM/sOBM monoclonal antibodies can be converted into human-type using the~~By CDR- grafting ~~technique~~method (Winter and Milstein: Nature 349, 293- to 299, 1991) ~~by implanting its~~, that is a method in which a CDR regions (CDR-1, 2 and 3) of the anti-human OBM/sOBM mouse monoclonal antibody with high affinity are transplanted into the CDR regions of human IgG, a humanized antibody can be obtained. ~~In the method~~ ③Moreover, (3) human peripheral blood ~~lymphocytes~~lymphocyte cells are ~~implanted~~transplanted into a severe combined immune deficiency (SCID) mouse. ~~Because the implanted~~Thus transplanted SCID mouse ~~can~~produces ~~produce~~a human antibod~~iesy~~ (Mosier D. ~~E.~~ E. *et al.*: Nature 335, 256- to 259, 1988~~;~~: Duchosal M. A. *et al.*: Nature 355, 258- to 262, 1992)~~, lymphocytes which can produce the human monoclonal antibodies having specificity to human OBM/ sOBM can be collected by screening SCID mouse immunized~~. The cells are sensitized with human OBM or sOBM as an antigen and screened. ~~The resulting lymphocytes~~Thereafter, a lymphocyte cell which produces a human-type monoclonal antibody specific to human OBM/sOBM can be extracted from the mouse. Then, as in the case of the above method for preparing a human-type antibody (1), the obtained lymphocyte cells are fused with K_6H_6/B_5 (ATCC CRL1823) ~~which is a heterohybridoma~~, a hetero hybridoma of mouse and human, ~~according to~~and then the obtained hybridomas are screened. Then, a hybridoma which

produces the target human-type monoclonal antibody can be obtained. By culturing the thus obtained hybridoma, the ~~procedure described above for the~~ target human ~~antibodies in the method ①. The resulting hybridomas are screened to obtain hybridomas which can produce the objective human~~-type monoclonal ~~antibodies. The thus obtained hybridomas are cultured to produce~~antibody can be produced in large quantities. After purifying them in the same manner as described above, large amounts of ~~the objective human monoclonal antibodies. The antibodies~~pure products thereof can be ~~purified by the above mentioned purification method.~~obtained. ~~In addition, it is possible to produce~~Furthermore, a recombinant human-type monoclonal ~~antibodies~~antibody can be produced in large ~~amounts~~quantities by constructing a cDNA library from ~~the~~said hybridoma which ~~can produce~~produces the ~~objective human monoclonal antibodies to obtain a gene (cDNA) encoding the objective~~target human-type monoclonal ~~antibodies by~~antibody, cloning the cDNA which encodes the target human-type monoclonal antibody, in~~se~~corporating ~~this gene~~ said cDNA into ~~a~~an ~~suitable~~appropriate expression vector by ~~using genetic engineering techniques~~gene engeneering, and expressing the ~~monoclonal antibodies~~antibody in ~~host cells such as various~~a variety of animal cells, insect cells~~,~~ or ~~Escherichia~~*E. coli*~~.~~ as a host. ~~A large amounts of purified human monoclonal antibodies can be obtained by purifying from the resulting~~After purifaction of the antibody from said culture ~~supernatant by the purification methods mentioned above~~according to the method as described above, a large amout of pure human-type monoclonal antibody can be obtained.

~~The antibodies which can neutralize~~Among the anti-OBM/sOBM monoclonal antibodies obtained by the above method, moreover, an antibody which neutralizes the biological activity of OBM/sOBM can be obtained ~~from the anti-OBM/sOBM monoclonal antibodies produced according to this method~~. ~~The~~These antibodies which neutralize the biological activity of OBM/sOBM are expected ~~to be useful~~ as medicaments, particularly agents for ~~the treatment~~ preventing and/or ~~prevention of~~treating bone metabolism abnormality ~~because of their capability of blocking in vivo~~, since they can inhibit the biological action (an activity ~~of OBM/sOBM, specifically the capability of preventing the induction~~ to promote osteoclast formation) of OBM/sOBM in a living body. The activity of the anti-OBM/sOBM antibod~~ies~~y to neutralize the

biological activity of OBM or sOBM can be ~~measured by determining the~~determined as an activity to ~~suppress~~inhibit osteoclast formation in ~~the~~an *in vitro* system for examining osteoclast formation. ~~Specifically~~As *in vitro* assay systems, the following three methods can be used. That is, *in vitro* culture systems for examining osteoclastogenesis ~~culture system can be given~~include: ~~①~~ (1) a co-culture system of a mouse ~~osteoblastic~~osteoblast-like stromal cell ~~strain~~line, ST~~2 cells,~~2, and mouse spleen cells in the presence of the active-form of vitamin D_3 and dexamethasone~~,~~; ~~②~~(2) a co-culture system ~~comprising~~in which OBM ~~expressing~~is expressed on a monkey kidney cell ~~strain~~line, COS-7, ~~immobilizing the OBM-expressing cells~~and fixed with formaldehyde, and ~~culturing mouse spleen cells on those~~then mouse spleen cells are cultured on the cells in the presence of M-CSF~~, and ③ a culture system of mouse spleen cells~~; and (3) a system of culturing mouse spleen cell in the presence of recombinant sOBM and M-CSF~~. The~~; however, other systems can be also used. When an anti-OBM/sOBM antibody is added to such a culture system in various concentration and its effect on osteoclastogenesis-~~inhibitory~~ is examined, an activity ~~of the anti-OBM/sOBM antibodies can be measured by adding the anti~~ of the anti-OBM/sOBM ~~antibodies at various concentrations to these culture systems and investigating their effects on osteoclast formation. The~~antibody to inhibit osteoclastogenesis ~~inhibitory~~ can be measured. Also, the activity ~~of~~ of the anti-~~OBM/sOBM antibodies can also be evaluated by measuring their~~-OBM/sOBM antibody to inhibit osteoclastogenesis can be determined as an activity to suppress bone resorption-~~inhibitory activity utilizing~~ *in vivo* using an experimental ~~animals in vivo. Especially, ovariectomized~~ animal ~~model is given as an~~. That is, there is an animal model ~~with progressive osteoclast formation. The osteoclastogenesis-inhibitory activity of the anti-OBM/sOBM antibodies can be determined by administering the~~, an overiectomized mouse, in which osteoclastogenesis is increased. An anti-~~OBM/sOBM antibodies to such~~ -OBM/sOBM antibody is administered to such a kind of experimental ~~animals and evaluating the suppression of~~animal, and an activity to inhibit bone resorption ~~(a~~ (an activity to reinforce bone mineral density ~~increasing activity~~) is measured. Thereby, an activity of the anti-OBM/sOBM antibody to inhibit osteoclastogenesis can be determined.

The ~~thus~~-obtained ~~antibodies capable of neutralizing~~antibody, which neutralizes the ~~OBM/sOBM~~ biological activity ~~are~~of OBM/sOBM is useful ~~in~~as a medicament,

particularly as a pharmaceutical ~~compositions, particularly pharmaceutical compositions to prevent~~ composition for preventing and/or treating bone metabolism abnormality, or as ~~antibodies for~~ an antibody used in immunological diagnosis of such ~~diseases~~a disease. The ~~preparations~~antibody of the present invention can be prepared in a formulation, and administered orally or parenterally. A formulation comprising the antibod~~ies~~y of the present invention ~~can be~~is administered ~~either orally or non-orally. Such preparations can be~~ safely ~~administered~~ to humans or animals as a pharmaceutical ~~compositions~~composition comprising the antibody which ~~contain the antibodies recognizing~~recognizes OBM and/or sOBM as an active ~~component~~ingredient. ~~As the forms~~Illustrative examples of the formulation of the pharmaceutical composition~~,~~ ~~injection agents including intravenous~~ include injectable solutions such as drip, suppository ~~agents~~, nasogastric agent, sublingual ~~agents, percutaneous absorption agents,~~agent and transdermal egent. Since the ~~like are given. Because~~ monoclonal ~~antibodies are macromolecule proteins, they not only readily adhere~~antibody has a high molecular weight, its adsorption to ~~a~~ glass containers such as ~~a~~ vial ~~or a~~and syringe tube is significant. Furthermore, ~~but also are~~the antibody is unstable and easily ~~denatured by~~inactivated due to various physicochemical factors such as heat, pH, ~~or~~and humidity. ~~Therefore~~Thus, to stably formulate the ~~preparations should be stabilized by the addition of stabilizers~~antibody, stabilizer, pH adjusters, ~~buffering agents~~buffer, solubilizing agents, ~~or detergents~~surfactant and the like are added thereto. ~~As~~Illustrative examples of the ~~stabilizers,~~stabilizer include amino acids such as glycine and alanine, saccharides such as dextran 40 and mannose, and sugar alcohols such as sorbitol, mannitol~~,~~ and ~~xylytol can be given~~xylitol. These ~~stabilizers~~ may be used ~~either individually or~~ in combinations of two or more. ~~The amount of~~These stabilizers ~~to be added is~~are preferably ~~from~~added in an amount which is 0.01 to 100 times, particularly ~~preferably from~~ 0.1 to 10 times, as much as the ~~amount~~weight of the antibody. ~~The~~By addition of these stabilizers, ~~increases~~the storage stability of liquid ~~preparations~~formulation or ~~lyophilized products thereof~~freeze-dried formulation can be improved. ~~Phosphate buffers and citrate buffers are given as~~Illustrative examples of ~~the buffering agents~~buffer include phosphate buffer and citricate buffer. The ~~buffering agents not only adjust~~buffer adjusts the pH of ~~the liquid preparations or~~an aqueous ~~solutions obtained by re~~formulation or a

reconstituted solution of freeze-~~dissolving the lyophilized products thereof~~dried formulation, ~~but also increase~~and thereby contributes to the stability and solubility of the antibody therein. ~~It is desirable to add the buffering agent in an~~The amount ~~to make from~~of the buffer is preferably, for example, 1 ~~mM~~ to 10 mM ~~concentration of the liquid preparation or of the~~in an aqueous formulation or a reconsituted solution ~~prepared from the lyophilized product~~of freeze-dried formulation. ~~Polysolbate~~The surfactant is preferably polysorbate 20, ~~Pulluronic~~PLURONIC® (BASF Co., Co.) F-~~68,~~68 and polyethylene glycol ~~are given as examples of the detergent. A~~, particularly ~~preferred example is Polysolbate 80.~~preferably polysorbate 20. These ~~detergents~~ may be used ~~either individually or~~ in combinations of two or more. ~~Macromolecule proteins such as~~A protein having high molecular weight like an antibody is ~~easily adhere~~liable to adsorb to glass ~~containers~~or resin, which a container is made of. ~~Adherence to containers~~However, by addition of a surfactant adsorption of the antibody ~~in a liquid preparation or~~to a container in an aqueous formulation or a reconsituted solution ~~prepared by re~~of freeze-~~dissolving a lyophilized product~~dried formulation can be prevented ~~by adding such detergents at a concentration from~~. The surfactant is preferably added in an amount of 0.001 to 1.0% of the weight of an aqueous formulation or a reconsituted solution of freeze-dried formulation. The ~~preparations~~formulation comprising the antibod~~ies~~y of the present invention can be ~~obtained~~prepared by ~~adding stabilizers~~addition of the stabilizer, ~~buffering agents, or agents which prevent~~buffer and adsorption-preventing agent as described above. ~~When the preparations are~~Particularly, when it is used as ~~injection agents~~an injectable formulation for ~~medication~~medical applications or ~~for~~treating animals, ~~such injection agents should preferably have an~~acceptable osmotic pressure ratio ~~of~~is preferably 1 ~~to~~or 2. The osmotic pressure ratio can be adjusted by increasing or decreasing ~~the amount of~~ sodium chloride ~~when making the preparations~~in formulation. The ~~amount~~content of ~~an~~the antibody in ~~a~~the ~~preparation~~formulation can be ~~suitably~~ adjusted ~~depending~~appropriately, dependent on the disease to be treated with said formulation, ~~route of~~ administration~~,~~ route and the like. ~~A~~The dose of ~~a~~the human-type antibody administered to humans ~~may be changed depending~~depends on the affinity of the antibody to human OBM/sOBM, ~~especially,~~that ~~on~~is, the dissociation constant (Kd value) of the anbibody to human OBM/sOBM. The higher the affinity is (or the lower

the Kd value is), the smaller the ~~Kd value), the less the~~ dose that is required to ~~be administered to humans to obtain a certain~~exhibit medicinal ~~effect~~benefits. ~~Because a human-type antibody has a long~~Furthermore, since the half-life time of human-type antibodies in human blood ~~of~~is about 20 days, ~~it is sufficient to administer it~~the human-type antibody can be administered to humans ~~at~~in a dose of about 0.1- to 100 mg/kg at least once ~~or more in a~~within 1- to 30 ~~day period~~days, for example.

BRIEF DESCRIPTION OF THE DRAWINGS

~~Figure~~Fig. 1 shows the results of SDS-PAGE of mouse OBM protein of Example 3 of the present invention ~~obtained in Example 3.~~, wherein:

~~<Explanation of symbols>~~

(A): Lane 1: ~~M~~molecular weight ~~markers~~marker,

Lane 2: ~~A~~ partially purified ~~sample (~~fraction eluted with Gly-HCl (pH 2.0) ~~elution fraction) obtained~~, which was derived from ST2 cells cultured in the presence of the active-form of vitamin D_3 and dexamethasone~~.~~,

Lane 3: ~~A~~ partially purified ~~sample (~~fraction eluted with Gly-HCl (pH 2.0) ~~elution fraction) obtained~~, which was derived from ST2 cells cultured in the absence of the active-form of vitamin D_3 and dexamethasone~~.~~,

(B): Lane 1: ~~M~~molecular weight ~~markers~~marker,

Lane 2: ~~M~~mouse OBM protein (Example 3) of the present invention ~~after purification by reverse~~purified with reversed phase high performance liquid chromatography ~~(Example 3).~~

~~Figure~~Fig. 2 shows the results of ~~the~~ binding ~~assay~~experiment of the ^{125}I-labeled OCIF to ~~osteoblastic~~an osteoblast-like stromal cells, ST2, in Example 4.

~~Figure~~Fig. 3 shows the osteoclastogenesis ~~formation capability~~-supporting abctivity of ~~osteoblastic~~osteoblast-like stromal ~~cells~~cell line, ST~~2~~2, ~~from~~with different ~~generations~~passage numbers, in Example 5(1)~~.~~, wherein:

~~<Explanation of symbols>~~

1: ~~Ability~~ osteoclastogenesis-supporting activity of ST2 cells ~~from about~~with a passage number of around 10^{th} ~~subculture to support osteoclast formation.~~'s,

2: ~~Ability~~ osteoclastogenesis-supporting activity of ST2 cells ~~from about~~with a passage number of around 40^{th} ~~subculture to support osteoclast formation~~'s.

~~Figure~~Fig. 4 shows a change ~~with the passage of time~~ in expression of the protein of the present invention on ~~the~~an osteoblast-like stromal cell membrane ~~of osteoblastic stromal~~, said cells were cultured in the presence of an active-form of vitamin D_3 and dexamethasone, with passage of time, in Example 5(2).

~~Figure~~Fig. 5 shows a change ~~with the passage of time~~ in ~~osteoclast formation~~osteoclastogenesis in the co-culture system, with passage of time, of Example 5 (2).

~~Figure~~Fig. 6 shows ~~the inhibitory effect on osteoclast formation~~osteoclastogenesis-inhibiting effects when OCIF was treated ~~with OCIF for different culture~~only during various culturing periods ~~during~~in the co-culture period ~~in~~of Example 5(3).

~~Figure~~Fig. 7 shows the results of ~~a~~ crosslinking ~~test~~experiment of the ^{125}I-labeled OCIF with the protein of the present invention, in Example ~~6.~~6, wherein:

~~<Explanation of symbols>~~

Lane 1: ^{125}I-labeled OCIF-CDD1,

Lane 2: ~~:~~ sample resulting from crosslinking of ^{125}I-labeled OCIF-CDD~~1 crosslinked with ST2 cells~~1 with an ST2 cell line,

Lane 3: ~~^{125}I-labeled OCIF-CDD1 crosslinked~~ sample resulting from crosslinking an ST2 cell in the presence of a 400-fold ~~excess~~higher concentration of unlabeled OCIF than that of ^{125}I-OCIF.

~~Figure~~ Fig. 8 shows the results of SDS-PAGE in Example ~~9.~~9, wherein:

~~<Explanation of symbols>~~

Lane 1: ~~Proteins of pOBM291 transfected~~ precipitate resulting from immuno precipitation of the protein of COS-7 cells ~~immonoprecipitated in the absence of~~transfected with pOBM291 without OCIF,

Lane 2: ~~Proteins of pOBM291 transfected~~ precipitate resulting from immuno precipitation of the protein of COS-7 cells transfected with pOBM291 with OCIF.

~~immunoprecipitated in the presence of OCIF~~

~~Figure~~Fig. 9 shows the results of ~~analysis of~~ binding ~~capability~~experiment of ^{125}I-labeled OCIF to COS-7 cells transfected with pOBM291 in Example ~~10.~~10, wherein:

~~<Explanation of symbols>~~

Lanes 1 and 2: ~~The~~ amount of ~~the~~ ^{125}I-labeled OCIF ~~binding~~bound to COS-7 cells transfected with pOBM~~291~~291,

Lanes 3 and 4: ~~The~~ amount of ~~the~~ ^{125}I-labeled OCIF ~~binding~~bound to COS-7 cells transfected with pOBM291 in the presence of a 400-fold ~~excess~~higher concentration of unlabeled OCIF than that of ^{125}I-OCIF.

~~Figure~~Fig. 10 shows the results of ~~a~~ crosslinking ~~test~~experiment using ~~OCIF~~ ^{125}I-labeled ~~with~~ ^{125}IOCIF in Example ~~11.~~11, wherein:

~~<Explanation of symbols>~~

Lane 1: ^{125}I-labeled OCIF,

Lane 2: sample resulting from crosslinking of ^{125}I-labeled OCIF ~~crosslinked~~ with COS-7 cells transfected with pOBM~~291~~291,

Lane 3: sample resulting from crosslinking of ^{125}I-labeled OCIF ~~crosslinked~~ with COS-7 cells transfected with pOBM291 in the presence of a 400-fold ~~excess~~higher concentration of unlabeled OCIF than that of ^{125}I-OCIF.

~~Figure~~Fig. 11 shows the results of ~~a~~ Northern ~~B~~blot in Example ~~12.~~12, wherein:

~~<Explanation of symbols>~~

Lane 1: RNA ~~originating~~derived from ST2 cells cultured ~~without addition~~in the absence of ~~V~~vitamin D and dexamethasone,

Lane 2: RNA ~~originating~~derived from ST2 cells cultured ~~with~~in the ~~addition~~presence of ~~V~~vitamin~~d~~ D and dexamethasone.

~~Figure~~Fig. 12 shows ~~the~~ OCIF- binding ~~capability~~ability of ~~the~~a proteins in the conditioned medium ~~at various~~when the concentration of OCIF ~~concentrations~~was varied in Example ~~13~~14-(2)., wherein:

~~<Explanation of symbols>~~

○: pCEP~~4~~4,

●: pCEP sOBM.

~~Figure~~Fig. 13 shows the OCIF- binding ~~capability~~ability of the protein in the conditioned medium ~~at various proportions~~when the amount of the conditioned medium was varied in Example ~~13~~14-(2)~~.~~, wherein:

~~<Explanation of symbols>~~

~~○: PCEP4~~

○: pCEP4,

●: ~~P~~pCEP sOBM.

~~Figure~~Fig. 14 shows the results of SDS-PAGE of a fusion protein ~~consisting~~ of thioredoxin and mouse OBM expressed in ~~Escherichia~~*E. coli*, in Example ~~14~~15-(2)~~.~~, wherein:

~~<Explanation of symbols>~~

Lane 1: ~~M~~molecular weight ~~markers~~marker,

Lane 2: ~~S~~soluble protein fractions ~~originating~~derived from GI724/pTrxFus,

Lane 3: ~~S~~soluble protein fractions ~~originating~~derived from GI724/pTrxOBM~~25~~25.

~~Figure~~Fig. 15 shows ~~the~~ OCIF- binding ~~capability at various proportions~~abilities when the amount of the soluble protein fractions were varied in Example ~~14~~15-(3)~~.~~, wherein:

~~<Explanation of symbols>~~

□: GI724/pTrxFus,

○: GI724/pTrxOBM~~25~~25.

~~Figure~~ Fig. 16 shows the OCIF- binding ~~capability~~abilities of the soluble protein fractions (1%) ~~at various~~ when the concentrations of OCIF was varied in Example ~~14~~15-(3)~~.~~, wherein:

~~<Explanation of symbols>~~

□: GI724/pTrxFus,

○: GI724/pTrxOBM~~25~~25.

~~Figure~~Fig. 17 shows the results of inhibition of the specific binding ~~to OCIF~~ of the mouse ~~OBM~~protein obtained by expressieng ~~of~~the mouse OBM cDNA of the present invention and ~~purification or~~purifying (mouse OBM) and the purified natural ~~OCIF~~-type OCIF binding protein to OCIF, by ~~a rabbit~~an anti-mouse OBM rabbit antibody~~.~~, wherein:

~~<Explanation of symbols>~~

1: ~~Purified OBM prepared by expression of the cDNA in the presence of the~~ purified recombinant OBM treated with an antibody~~, OBM~~ + ^{125}I-OCIF,

2: ~~The~~ the purified natural-type protein ~~in the presence of the~~treated with an antibody + ^{125}I-OCIF,

3: ~~Mouse OBM prepared by expression of the cDNA in the absence of the~~ purified recombinant OBM untreated with an antibody~~, mouse OBM~~ + ^{125}I-OCIF,

4: ~~The~~ the purified natural-type protein ~~in the absence of the~~untreated with an antibody + ^{125}I-OCIF,

5: 3 + unlabeled OCIF (400-fold ~~more~~higher concentration than that of ^{125}I-OCIF),

6: 4 + unlabeled OCIF (400-fold ~~more~~higher concentration than that of ^{125}I-OCIF).

~~Figure~~Fig. 18 shows the results of SDS-PAGE of human OBM protein expressed by the cDNA of the present invention~~.~~, wherein:

~~<Explanation of symbols>~~

Lane 1: ~~M~~molecular weight ~~markers~~marker,

Lane 2: ~~Proteins of~~ precipitate resulting from immuno precipitation of the protein derived from COS-7 cells transfected with ~~phOBM (~~an expression vector (phOBM) containing ~~a~~the cDNA of the present invention~~), immunoprecipitated with a rabbit~~ by an anti-OCIF rabbit polyclonal antibody ~~in the absence of~~without OCIF,

Lane 3: ~~Proteins of~~ precipitate resulting from immuno precipitation of the protein derived from COS-7 cells transfected with ~~phOBM (~~an expression vector (phOBM) containing ~~a~~the cDNA of the present invention~~), immunoprecipitated with a rabbit~~ by an anti-OCIF rabbit polyclonal antibody ~~in the presence of~~with OCIF.

~~Figure~~Fig. 19 shows the results of ~~analysis of~~a binding experiment of OCIF to COS-7 cells transfected with ~~phOBM,~~ an expression vector (phOBM) containing ~~a~~the cDNA of the present invention~~.~~, wherein:

~~<Explanation of symbols>~~

Lane 1: COS-7 cells transfected with phOBM ~~and the addition of~~+ ^{125}I- OCIF~~.~~,

Lane 2: COS-7 cells transfected with phOBM ~~and the addition of~~+ ^{125}I- OCIF~~, in the presence of~~ + a 400-fold ~~more~~higher concentration of unlabeled OCIF than that of ^{125}I-OCIF.

~~Figure~~Fig. 20 shows the results of crosslinking experiment of human OBM~~, which is a~~ protein encoded by ~~a~~the cDNA of the present invention~~,~~ with ^{125}I-OCIF (monomer- type)~~.~~, wherein:

~~<Explanation of symbols>~~

Lane 1: ^{125}I-OCIF~~,~~

Lane 2: ~~The crosslinked products~~ sample resulting from crosslinking of ^{125}I-OCIF with ~~the~~a proteins on the membrane of COS-7 cells transfected with phOBM~~.~~,

Lane 3: ~~The crosslinked products~~ sample resulting from crosslinking of ^{125}I-OCIF with ~~the~~a proteins on the membrane of COS-7 cells transfected with ~~pHOBM,~~phOBM in the presence of a 400-fold ~~more~~higher concentration of unlabeled OCIF than that of ^{125}I-OCIF.

~~Figure~~Fig. 21 shows the OCIF- binding ~~capability~~ability of ~~the~~a protein (secrete~~d~~ory-~~form hOBM~~type human OBM) in the conditioned medium ~~at various~~when the concentration of OCIF ~~concentrations~~was varied in Example ~~23~~24-(2)~~.~~, wherein:

~~<Explanation of symbols>~~

○: ~~C~~conditioned medium of 293-EBNA cells transfected with pCEP4~~, which does~~4 vector not containing cDNA ~~encoding secreted~~which encodes the secretory-~~form~~type human OBM~~,~~

●: ~~C~~conditioned medium of 293-EBNA cells transfected with pCEPshOBM~~,~~ expression vector containing cDNA which ~~contains cDNA encoding secreted~~encodes the secretory-~~form~~type human OBM.

~~Figure~~Fig. 22 shows the OCIF- binding ~~capability~~ability of the protein (secrete~~d~~ory-~~form~~type human OBM) in the conditioned medium ~~at a specific OCIF concentration~~when the amount of the conditioned medium to be added was varied while ~~changing~~ the ~~amount~~concentration of ~~conditioned medium added~~OCIF was kept constant, in Example ~~23~~24-(2)~~.~~, wherein:

~~<Explanation of symbols>~~

○: ~~C~~conditioned medium of 293-EBNA cells transfected with pCEP4~~, which does~~4 vector not containing cDNA ~~encoding secreted~~which encodes the secretory-~~form~~type human OBM~~,~~

●: ~~C~~conditioned medium of 293-EBNA cells transfected with pCEPshOBM~~;~~ expression vector containing cDNA which ~~contains cDNA encoding secreted~~encodes the secretory ~~form~~type human OBM.

~~Figure~~Fig. 23 shows the results of SDS-PAGE of a fusion protein ~~consisting~~ of thioredoxin and human OBM, expressed in ~~Escherichia~~*E. coli*~~.~~, wherein:

~~<Explanation of symbols>~~

Lane 1: ~~M~~molecular weight ~~markers~~marker,

Lane 2: ~~S~~soluble protein fractions ~~originating~~derived from ~~Escherichia~~*E. coli* GI724/pTrxFus,

Lane 3: ~~S~~soluble protein fractions ~~originating~~derived from ~~Escherichia~~*E. coli* GI724/~~pTrxhOBM~~pTrxOBM.

~~Figure~~Fig. 24 shows the ~~OCIF binding capability~~ability of ~~the~~a fusion protein ~~consisting of thioredoxin and human OBM to OCIF, when the~~ to bind OCIF when the amount of ~~the~~ soluble protein fraction ~~originating from Escherichia~~containing the fused protein of thioredoxin and human OBM expressed in *E. coli* ~~including the fusion protein added~~ was varied, in Example ~~24~~25-(3)~~.~~, wherein:

~~<Explanation of symbols>~~

○: ~~S~~soluble protein fractions ~~originating~~derived from ~~Escherichia~~*E. coli* GI724/pTrxFus,

●: ~~S~~soluble protein fractions ~~originating~~derived from ~~Escherichia~~*E. coli* GI724/pTrxshOBM.

~~Figure~~Fig. 25 shows the ~~OCIF binding capability~~ability of the fusion protein of thioredoxin and human OBM in a soluble protein ~~fractions originating from Escherichia~~fraction of *E. coli* to bind OCIF ~~in various concentrations~~when the concentration of OCIF was varied, in Example ~~24~~25-(3~~).~~), wherein:

~~<Explanation of symbols>~~

○: ~~S~~soluble protein fractions ~~originating~~derived from ~~Escherichia~~*E. coli* GI724/pTrxFus

●: ~~S~~soluble protein fractions ~~originating~~derived from ~~Escherichia~~*E. coli* GI724/pTrxshOBM.

~~Figure~~Fig. 26 shows the results of ~~quantifying~~measurement of human OBM and human sOBM by ~~the~~ sandwich ELISA ~~method~~ using ~~the rabbit~~an anti-human OBM/sOBM rabbit polyclonal antibody of the present invention~~.~~, wherein:

~~<Explanation of symbols>~~

□: ~~H~~human OBM,

●: ~~H~~human sOBM.

~~Figure~~Fig. 27 shows the results of ~~quantifying~~measurement of human OBM and human sOBM by ~~the~~ sandwich ELISA ~~method~~ using ~~the~~an anti-human OBM/sOBM monoclonal antibod~~ies~~y of the present invention~~.~~, wherein:

~~<Explanation of symbols>~~

□: ~~H~~human OBM,

●: ~~H~~human sOBM.

~~Figure~~Fig. 28 shows the results of ~~quantifying~~measurement of mouse OBM and mouse sOBM by ~~the~~ sandwich ELISA ~~method~~ using ~~the~~an anti-human OBM/sOBM monoclonal antibod~~ies~~y of the present invention ~~which~~, said antibody has cross-~~react~~-reactivity to both mouse OBM and mouse sOBM~~.~~, wherein:

~~<Explanation of symbols>~~

□: ~~M~~mouse OBM,

●: ~~M~~mouse sOBM.

~~Figure~~Fig. 29 shows ~~the~~an activity of ~~the~~a fusion protein ~~consisting~~ of thioredoxin and mouse OBM to ~~stimulate~~promote the formation of human osteoclast-like ~~cell formation.~~cells

~~Figure~~Fig. 30 shows ~~the~~ suppression of ~~the~~vitamin D_3-stimulated bone resorption by an anti-OBM/sOBM antibody ~~of the bone resorption activity stimulated by vitamin D_3~~.

~~Figure~~Fig. 31 shows ~~the~~ suppression of ~~the anti-OBM/sOBM antibody of the bone resorption activity stimulated by~~ prostaglandin E_2 (PGE_2)-stimulated bone resorption by an anti-OBM/sOBM antibody.

~~Figure~~Fig. 32 shows ~~the~~ suppression ~~by the anti-OBM/sOBM antibody of the bone-resorbing activity stimulated by~~ parathyroid hormone (PTH)-stimulated bone resorption by an anti-OBM/sOBM antibody.

~~Figure~~Fig. 33 shows ~~the~~ suppression ~~by the~~of interleukin 1 α (IL-1)-stimulated bone resorption by an anti-OBM/sOBM antibody ~~of the bone-resorbing activity stimulated by interleukin 1 α (IL-1)~~.

BEST MODE FOR ~~CARRYING OUT~~PRACTICING THE INVENTION

[Examples]

The present invention ~~will be described~~is explained in more detail ~~by way of examples which are given for~~with reference to the ~~purpose of illustration of~~following Examples. However, these Examples are only exemplary and shall not limit the present invention ~~and are not limiting thereof~~ thereto in any way ~~of the remainder of the disclosure~~.

~~<~~[Example 1~~>~~]

Pr~~epara~~oduction of the ~~p~~Protein of the ~~p~~Present ~~i~~Invention

(1) Large- ~~s~~Scale ~~cultivation~~ Culture of ST2 ~~c~~Cells

Mouse ~~osteoblastic~~osteoblast like stromal cell line, ST~~2~~2, (~~RIKEN CELL BANK~~Riken Cell Bank, RCB0224) was cultured ~~using~~with ~~α~~α-MEM medium containing 10% ~~fetal~~ bovine fetal serum. ~~ST2 cells~~After cultured to ~~confluence~~become confluent in a 225 -cm^2 T flask for adherent ~~cell culture~~ cells, ST2 cells were treated with trypsin ~~and harvested~~, stripped from the T flask. ~~After washing~~, ~~the cells were~~washed, and then transferred to five of 225 -cm^2 T flask~~s~~. After ~~the~~ addition of 60 ml of ~~α~~α-MEM medium containing 10^{-8} M of the active-form of vitamin D_3 (~~C~~calcitriol), 10^{-7} M dexamethasone~~,~~ and ~~10%~~bovine fetal ~~bovine~~ serum, the resulting cells ~~in each flask~~ were cultured ~~for 7-10 days~~ in a CO_2 incubator for 7 to 10 days. The cultured ST2 cells were ~~harvested~~recovered using a cell scraper and stored at -80°C until use.

(2) Preparation of ~~m~~Membrane ~~f~~Fraction and ~~s~~Solubilization of ~~m~~Membrane-~~b~~Bound ~~p~~Proteins

To ~~the~~ ST2 cells (~~volume,~~amount: about 12 ml) described in Example 1-(1)~~,~~ which were cultured ~~using eighty~~with 80 of 225 -cm^2 T flasks, ~~was added three times~~

~~the~~a 3-fold volume (36 ml) of 10 mM Tris-~~HCl~~hydrochloric acid buffer (pH 7.2) containing protease inhibitors (2 mM APMSFP, 2 mM EDTA, 2 mM o-~~-~~phenanthroline, 1 mM leupeptin, 1 ~~μg~~μg/ml pepstatin A~~,~~ and 100 units/ml aprotinin) were added. After these cells were vigorously ~~agitating~~agitated by use of a vortex mixer for 30 seconds ~~using a voltex mixer~~, ~~the cells~~they were ~~allowed~~left to stand on ice for 10 minutes ~~on ice~~. ~~The cells were homogenized using~~Using a homogenizer (~~DOUNCE TISSUE GRINDER~~Dounce Tissue Grinder, A syringe, ~~WHEATON~~Wheaton ~~SCIENTIFIC~~Scientific Co., Ltd.), these cells were crushed. ~~The same~~To the crushed cell solution, an equal volume (48 ml) of 10 mM Tris-~~HCl~~hydrochloric acid buffer (pH 7.2) containing the above ~~mentioned~~ protease inhibitors, 0.5 M sucrose, 0.1 M potassium chloride, 10 mM magnesium chloride~~,~~ and 2 mM calcium chloride was added ~~to the homogenized cells~~. ~~After stirring, the~~The obtained mixture was agitated and then centrifuged at 600 x g at 4°C for 10 minutes ~~at 4°C~~. Through this centrifugation, ~~thereby separating~~cell nuclei and ~~non-homogenized~~uncrushed cells were separated as ~~precipitate~~precipitated fractions. ~~The~~A supernatant obtained ~~by the centrifuge~~after centrifugation was further centrifuged at 150,000 x g at 4°C for 90 minutes ~~at 4°C~~, ~~to obtain~~and membrane fractions of the ST2 cells were obtained as ~~precipitate~~precipitated fractions. ~~Eight~~To the membrane fractions, 8 ml of 10 mM Tris-~~HCl~~hydrochloric acid buffer (pH 7.2) containing the above ~~mentioned~~ protease inhibitors, 150 mM of sodium chloride, and 0.1 M sucrose was added ~~to this membrane fraction. After the addition of~~, and then 200 ~~μl~~μl of 20% CHAPS (3-[(3-cholamidopropyl)~~-dimethylamonio-~~dimethylammonio]~~-~~1~~-~~propanesulfonate, ~~Sigma~~aigma Co.~~)~~, ~~the~~Ltd.) was added. The mixture was ~~stirred~~agitated at 4°C for 2 hours ~~at 4°C~~. The~~is mixture~~solution was ~~then~~ centrifuged at 150,000 x g at 4°C for 60 minutes ~~at 4°C~~, ~~to obtain~~and the resulting supernatant was obtained as a solubilized membrane fraction.

<~~[~~Example 2~~>~~]

Purification of the ~~p~~Protein of the ~~p~~Present ~~i~~Invention

(1) Preparation of OCIF-~~i~~Immobilized ~~a~~Affinity ~~c~~Column

~~After replacing iso-propanol~~Isopropanol in a ~~HiTrap~~HITRAP® NHS-activated column (1 ml, ~~manufactured by~~ Pharmacia Co., Ltd.) was substituted with 1 mM hydrochloric acid, and 1 ml of 0.2 M $NaHCO_3$/0.5 M NaCl (pH 8.3) solution ~~(pH 8.3)~~

containing 13.0 mg of recombinant OCIF prepared ~~by the~~in accordance with a method ~~of~~described in WO 96/26217 was added to the column using a syringe (5 ml, ~~manufactured by~~ Terumo ~~Corp.~~Corporation)~~,~~. After the column was allowed to ~~effect~~undergo a coupling reaction at room temperature for 30 minutes~~. The column was fed with~~, 3 ml of 0.5 M ethanolamine/0.5 M NaCl (pH 8.3) and 3 ml of 0.1 M acetic acid/0.5 M NaCl (pH 4.0) were loaded on the column alternately three times each in ~~turn~~total so as to inactivate excessive activated groups. Then, ~~then~~ the ~~solution~~mobile phase of the column was ~~replaced~~substituted again with 0.5 M ethanolamine/0.5 M NaCl (pH 8.3)~~. After allowing~~ and then left to stand at room temperature for 1 hour. Thereafter, the resulting column was washed twice ~~alternately~~ with 0.5 M ethanolamine/0.5 M NaCl (pH 8.3) and 0.1 M acetic acid/0.5 M NaCl (pH 4.0)~~, and~~ and then the ~~solution~~mobile phase was ~~then replaced~~substituted with 50 mM Tris/1 M NaCl/0.1% CAHPS buffer (pH 7.5).

(2) Purification of the ~~p~~Protein of the ~~present invention using~~Present Invention by an OCIF-~~i~~Immobilized ~~a~~Affinity ~~c~~Column

~~The purification~~Purification of ~~the~~ OCIF- binding protein was carried out at 4°C~~,~~ unless otherwise ~~indicated~~stated. The above~~-mentioned~~ OCIF-immobilized affinity column was equilibrated with 10 mM Tris-~~hydrochloride~~hydrochloric acid buffer (pH 7.2) ~~to which~~containing the protease inhibitors described in Example 1-(2), 0.15 M sodium chloride~~,~~ and 0.5% CHAPS ~~were added~~. ~~About~~To this column, about 8 ml of the solubilized membrane fraction described in Example 1-(2) was applied ~~to the column~~ at a flow rate of 0.01 ml/min~~ute~~. ~~Then, the~~The column was washed with the above 10 mM Tris-~~hydrochloride~~hydrochloric acid buffer (pH 7.2) ~~to which~~containing the ~~above-mentioned~~ protease inhibitors, 0.15 M sodium chloride~~,~~ and 0.5% CHAPS ~~was added, for 100 minutes~~ at a flow rate of 0.5 ml/~~minute~~min for 100 minutes. ~~Next~~Then, the proteins ~~adsorbed to~~were eluted from the column ~~was eluted~~ with 0.1 M glycine-~~hydrochloride~~hydrochloric acid buffer (pH 3.3) containing the above protease inhibitors, 0.2 M sodium chloride~~,~~ and 0.5% CHAPS ~~for 50 minutes~~ at a flow rate of 0.1 ml/~~minute~~min for 50 minutes. ~~In the same manner~~Similarly, ~~the proteins adsorbed to the column was eluted with~~a 0.1 M sodium citrate buffer (pH 2.0) containing ~~the~~said protease inhibitors, 0.2 M sodium chloride~~,~~ and 0.5% CHAPS ~~for 50 minutes~~was fed to

the column at a flow rate of 0.1 ml/~~minute~~min for 50 minutes so as to elute proteins adsorbed to the column. The eluates ~~was collected in~~ were fractionated as 0.5 ml/fraction each. The fractions. ~~Each fraction was~~ were immediately neutralized by ~~the~~ addition of a 2M Tris solution. The fractions ~~derived from the elution with these buffers (each fraction consisting~~the volume of the eluate was 1.0 to 5.0 ml ~~of eluate~~) eluted with the buffer were concentrated to 50 to 100 ~~μ~~μl using ~~Centricon-10 (manufactured by Amicon of U~~CENTRICON®-10 (Amersham Co.~~S.A~~, Ltd.). ~~OCIF was added to a portion of each~~Aliquots of the concentrated ~~fraction~~ fractions were subfractionated, and after addition of OCIF to the aliquots, they were immunoprecipitated with an anti-OCIF polyclonal antibody. ~~The~~After the precipitated fractions were treated with SDS ~~and~~, they were subjected to SDS-PAGE. ~~Fractions~~, and then a fraction (Fr. ~~No.~~ Nos. 3-10) ~~in which the~~showing a band of the protein ~~with specific binding ability~~having an activity to specifically bind OCIF ~~appeared were regarded~~was identified as the protein fractions of the present invention.

(3) Purification of the ~~p~~Protein of the ~~p~~Present ~~i~~Invention by ~~g~~Gel ~~f~~Filtration

The ~~concentrated~~ OCIF- binding protein ~~(the fractions obtain by the elution~~eluted with 0.1 M glycine ~~hydrochloride~~hydrochloric acid buffer (pH 3.3) and subsequently 0.1 M sodium citrate buffer (pH 2.0)~~) prepared~~ after purification and concentration in accordance with the method described in Example 2 (2) was ~~applied~~subjected to a ~~Superose~~SUPEROSE® 12 HR10/30 column (~~1.0 x 30 cm, manufactured by~~ Pharmacia Co., Ltd., 1.0 X 30 cm) ~~which was~~ equilibrated with 10 mM Tris-HCl, 0.5 M NaCl, and 0.5% CHAPS (pH 7.0) and developed ~~with~~using the above equilibration buffer as a mobile phase at a flow rate of 0.5 ml/min, and ~~each~~then fractions of 0.5 ml ~~fraction was~~were collected. The fractions containing the protein of the present invention (Fr. ~~Nos.~~ Nos. 27-32) ~~were~~was identified ~~according to~~and concentrated by means of CENTRICON®-10 (Amersham Co., Ltd.) in the same ~~method~~manner as described above. ~~Each of the fractions was concentrated using Centricon-10 (a product of Amicon).~~

(4) Purification by rReversed pPhase hHigh pPerformance lLiquid eChromatography

The above-mentioned OCIF- binding protein purified by the above gel filtration was appliedadded to a C_4 column (2.1 xX 250 mm, Vydac, USA) which was equilibrated with 0.1% trifluoroacetic acid (TFA) and 30% acetonitrile. The proteins bound to the column were elutedElution was carried out at a flow rate of 0.2 ml/min with linearthe gradients of acetonitrile concentration of from 30% to 55% for the first 50 minutes and then of from 55% to 80% during the nextfor another 10 minutes at a flow rate of 0.2 ml/min. PeaksThe peaks of eluted proteins were detected by measuring optical density at 215 nmmm. Proteins in the different peaks were analyzed to identify the fractions containingThe eluted protein of each peak was fractionated, and the peak of the protein of the present invention, and was identified. Thus, a highly purified protein of the present invention was obtained.

<[Example 3>]

SDS-PAGE of the pPurified pProtein of the pPresent lInvention

TheFirst, a solubilized membrane fraction prepared from ST2 cells which were cultured in the presence or absence of the active-form of vitamin D_3 was subjected to purificationpurified with the OCIF-immobilized affinity column. The as described above, and the purified preparationssamples were subjected to SDS-PAGE. As shown in FigureFig. 1(A), it was revealed that a major protein band with MW of about 30,000 to 40,000 was detected only in the purified preparationsample obtained from the ST2 cells which was cultured in the presence of the active-form of vitamin D_3, provingand that thea protein which specifically binds to OCIF, (i.e., the protein of the present invention) can be, is selectively concentrated and purified bywith the OCIF-immobilized affinity column. However, bands of several proteins (other thanin addition to the protein of the present invention), some other bands of proteins which non-specifically were nonspecifically biound to the carriers or, spacers or the like of the OCIF-immobilized column were also detected in both of the purified preparationssamples. These proteins other than the protein of the present invention were removed according to the above-described method by gel filtration and C_4 reversed phase chromatography as described above. The SDS-PAGE of the obtained highly purified protein of the present invention is

shown in ~~Figure~~ Fig. 1(B). The highly purified protein of the present invention was ~~found to be~~ electrophoretically homogeneous, and ~~had a~~the molecular weight ~~of~~thereof was about 30,000~~-~~ to 40,000.

~~<~~[Example 4~~>~~]

Examining the Binding ~~test~~ of OCIF to ~~o~~Osteoblasts

(1) Preparation of ^{125}I-~~l~~Labeled OCIF

OCIF was ^{125}I-labeled ~~with ^{125}I~~ by ~~the~~ Iodogen method. ~~Specifically~~More specifically, 20 ~~μ~~μl of 2.5 mg/ml Iodogen-chloroform solution was transferred to a 1.5 ml Eppendorf tube, and chloroform was evaporated ~~off~~ at 40°C~~, to obtain a tube coated with~~ so as to prepare an Iodogen-coated tube. ~~The~~After the tube was washed three times with 400 ~~μl~~μl of 0.5 M sodium phosphate buffer (Na-Pi, pH 7.0)~~. Five μl~~, 5 μl of 0.5 M Na-Pi (pH 7.0) was added ~~to the tube~~thereto. Immediately after ~~the addition of~~ 1.3 ~~μl~~μl (18.5 MBq) of Na-^{125}I solution (~~NEZ-033H20, manufactured by~~ Amersham Co.~~)~~, Ltd., NEZ-033H20) was added to the tube, 10 ~~μl~~μl of 1 mg/ml rOCIF solution (monomer type or dimmer type) was added ~~to the tube~~. ~~After mixing~~The obtained solution was agitated with a vo~~l~~rtex mixer, ~~the mixture was allowed~~and then left to stand at room temperature for 30 seconds. The solution was transferred to a tube containing 80 ~~μl of a solution of~~μlof 10 mg/ml potassium iodide ~~in~~and 0.5 M Na-Pi solution (pH 7.0), and 5 ~~μl~~μl of ~~a~~ phosphate buffered saline solution containing 5% bovine serum albumin, and ~~stirred~~then agitated. The~~is~~ ~~mixture~~solution was applied to a spin column (1 ml, G-25 fine, ~~manufactured by~~ Pharmacia Co., Ltd.) ~~which was~~ equilibrated with a phosphate buffered saline solution containing 0.25% bovine serum albumin and ~~the column was~~ centrifuged at 2,000 rpm for 5 minutes ~~at 2,000 rpm~~. ~~Four hundred μ~~After adding 400 μl of ~~a~~ phosphate buffered saline solution containing 0.25% bovine serum albumin ~~was added~~ to the fraction eluted from the column and ~~the mixture was stirred.~~ Asubsequently mixed, 2 ~~μ~~μl ~~of the aliquot was removed to measure~~aliquots were collected, and the radioactivity ~~using~~thereof was measured with a gamma counter. The radiochemical purity of the thus prepared ^{125}I-labeled OCIF solution was determined by measuring the radioactivity of a fraction precipitated ~~with~~by 10% TCA. ~~The~~Furthermore, the biological activity ~~of~~as OCIF the ^{125}I-labeled OCIF solution was ~~measured according to the~~determined in accordance with a method described in WO 96/26217. ~~The~~Moreover, the concentration

of ~~the~~ ^{125}I-labeled OCIF was measured by ~~the~~ ELISA ~~according to~~in the following ~~procedure~~manner.

(2) Measurement of ~~the concentration~~Concentration of ^{125}I-~~l~~Labeled OCIF by ELISA

~~One hundred~~100 ~~μ~~μl of 50 mM $NaHCO_3$ (pH 9.6) in which ~~the~~2 μg/ml of anti-OCIF rabbit polyclonal antibody described in WO 96/26217 was dissolved ~~to a concentration of 2 μg/ml~~ was added to each well of ~~a~~ 96-well ~~immuno-plate~~immunoplate (MaxiSorp™, ~~a product of~~ Nunc Co.~~). The plate was allowed~~, Ltd.) and left to stand at 4°C overnight ~~at 4°C~~. After ~~removing the~~this solution ~~by~~was ~~suction~~removed, 300 ~~μ~~μl of ~~Block Ace™~~BLOCKACE (Snow Brand Milk Products Co., Ltd.)/phosphate buffered saline ~~(25/75)~~ solution (25/75) was added to each well~~. The plate was~~ and then ~~allowed~~left to stand ~~for two hours~~ at room temperature~~.~~ for 2 hours. After ~~removing the~~this solution ~~by suction, the~~ was removed, each well~~s were~~ was washed three times with phosphate buffered saline solution (P-PBS) containing 0.01% ~~Polysorbate 80 (P-PBS).~~polysorbate 80. ~~Next~~Thereafter, 300 ~~μ~~μl of ~~Block Ace™~~BLOCKACE/phosphate buffered saline ~~(25/75)~~ solution ~~to which~~(25/75) containing ^{125}I-labeled OCIF sample or ~~the~~ standard OCIF ~~preparation was mixed,~~ was added to each well~~. The plate was then allowed~~ and left to stand ~~for two hours~~ at room temperature for 2 hours. After ~~removing the~~this solution ~~by~~was ~~suction~~removed, each well was washed six times with 200 ~~μ~~μl of P-PBS.~~One hundred μl~~ Then, 100 μl of ~~Block Ace™~~BLOCKACE (Snow Brand Milk Products Co., Ltd.)/phosphate buffered saline ~~(25/75)~~ solution (25/75) containing peroxidase -labeled ~~rabbit~~ anti-OCIF rabbit polyclonal antibody was added to each well~~. The plate was allowed~~ and left to stand ~~for two hours~~ at room temperature for 2 hours. After ~~removing the~~this solution ~~by~~was ~~suction~~removed, ~~the~~ each wells ~~were~~ was washed six times with 200 ~~μl~~μl of P-PBS. ~~Then, 100 μ~~Then, 100 μl of TMB solution (TMB Soluble Reagent, High Sensitivity, Scytek Co., Ltd.) was added to each well~~. After incubating~~ and then left to stand at room temperature for 2- to 3 minutes. Thereafter, 100 ~~μ~~μl of ~~stopping solution (~~Stopping Reagent~~,~~ (Scytek Co., Ltd.) was added to each well. ~~Absorbance~~The absorbance of each well at 490 nm was measured ~~at 490 nm using~~with a microplate reader. The concentration of the ^{125}I-labeled OCIF was ~~determined~~calculated from a calibration curve ~~prepared~~made by using ~~the~~ standard ~~preparation of~~ OCIF.

(3) Examining the Binding ~~test~~ of OCIF to ~~o~~Osteoblasts or ~~spleen~~Pancreas ~~c~~Cells

Mouse ~~osteoblastic~~osteoblast-like stromal cell line, ST~~2~~2, or ~~spleen~~mouse pancreas cells were suspended in ~~α~~α-MEM medium containing 10% bovine fetal ~~bovine~~ serum (FBS)~~, either~~ with or without 10^{-8} M of the active-form of vitamin D_3 (~~C~~calcitriol) and 10^{-7} M dexamethasone~~,~~ ~~to~~at a concentration of 4 ~~×~~X 10^4 cells/ml ~~(ST~~and 2 ~~cells) or 2~~ ~~×~~X 10^6 cells/ml ~~(spleen cells)~~, respectively. ~~Each cell suspension~~1 ml of this medium was ~~innoculated~~seeded ~~into~~in a 24~~-~~ well ~~micro plate~~microplate. ~~The~~After the cells were cultured ~~for 4 days in~~ in a CO_{2}~~2~~ incubator~~. After washing the cells~~ for 4 days and washed with ~~α~~α-MEM medium, 200 ~~μ~~μl of medium for the binding ~~test~~experiment (~~α~~α-MEM ~~to which~~medium containing 0.2% bovine serum albumin, 20 mM Hepes buffer~~,~~ and 0.2% NaN_3 ~~were supplemented)~~; further containing 20 ng/ml of the above-~~described~~ ^{125}I-labeled OCIF (monomer ~~form~~type or dimmer ~~form~~type)~~,~~ was added to each well. ~~To the wells for the measurement of non-specific binding~~Furthermore, 200 ~~μl~~μg/ml of the medium for the binding ~~test~~experiment containing 8 ~~μ~~μg/ml ~~of~~ rOCIF (400 ~~times~~-fold higher concentration) ~~in addition to 20 ng/ml of ^{125}I-labeled OCIF~~ was added~~. The~~ to other wells which were subjected to measurements of nonspecific binding. After the cells were cultured ~~for one hour~~ in a CO_{2}~~2~~ incubator ~~and~~for 1 hour, they were washed ~~3~~three times with 1 ml of ~~a~~ phosphate buffered saline solution. ~~In this procedure~~Since pancreas cells are floating cells, ~~spleen~~ cells in each well were washed ~~by centrifuging~~in the 24-well plate ~~in each washing step, because the spleen cells were non-adherent~~with centrifugation. After washing, 500 ~~μ~~μl of 0.1 N NaOH solution was added to each well and ~~the plate was allowed~~left to stand ~~for 10 minutes~~ at room temperature ~~to dissolve~~for 10 minutes. Thereby, the cells~~. The~~ were washed, and the amount of RI ~~in each well~~bound to the cells was measured ~~by~~with a gamma counter.

~~As shown in Figure 2,~~The ^{125}I-labeled OCIF did not bind ~~to~~ the cultured ~~spleen~~pancreas cells, but specifically bound only ~~to the osteoblastic~~osteoblast like stromal cells ~~which were~~ cultured in the presence of the active-form of vitamin D_3. ~~The results indicated~~Thereby, it was revealed that the protein of the present invention ~~is~~was a membrane -bound protein induced ~~by~~on the cell surface of osteoblast like stromal cells with the active-form of vitamin D_3 and dexamethasone ~~on osteoblastic stromal cells~~.

~~<~~[Example 5~~>~~]

Biological aActivity of the pProtein of the pPresent iInvention

~~(1) Osteoclasts formation supported by osteoblastic stromal cells~~

(1) Ability of Osteoblast Like Stromal Cells to Support Osteoclast Formation

The ~~osteoclasts~~ability of osteoblasts to support osteoclast formation ~~supporting capability of osteoblastic stromal cells~~ was ~~evaluated~~examined by measuring tartaric acid resistant acid phosphatase activity (TRAP activity) of the formed osteoclasts. ~~Specifically~~More specifically, ~~spleen~~mouse osteoblast-like stromal cell line, ST2, (5 X 10^3 cells/100 µl/well) (2 ~~x~~X 10^5 cells/100 ~~µ~~µl/well) and pancreas cells derived from a ddy mouse (8- to 12 weeks old) ~~and mouse osteoblastic stromal cells ST2 (5 x 10^3 cells/100 µl/well)~~ were suspended in ~~α~~α-MEM ~~to which 10^{-8}~~-medium containing 10% bovine fetal serum, 10^{-8} M of the active-form ~~vitamin D_3, 10^{-8}~~ of vitamin D_3 and 10^{-7} M dexamethasone~~,~~ and ~~10% fetal bovine serum were added~~seeded in a 96-well plate. ~~The~~After the cells were ~~innoculated into 96-well plates and~~ cultured in a CO_2 incubator for one week ~~in a CO_2 incubator. After washing,~~ each well was washed with phosphate buffered saline solution. Then, 100 ~~µ~~µl of ethanol/acetone (1:1) was further added to ~~each~~the ~~well,~~wells and ~~the cells were immobilized~~fixed at room temperature for ~~one~~1 minute. ~~After immobilization~~Then, 100 ~~µ~~µl of 50 mM ~~citrate~~citric acid buffer (pH 4.5) containing 5.5 mM p-nitrophenol phosphate and 10 mM sodium tartarate was added to each well. ~~After~~ and then allowed to react at room temperature for 15 minutes. After ~~of~~the reaction ~~at room temperature~~, 0.1 N NaOH solution was added to each well, and the absorbance at 405 nm was measured ~~using~~with a microplate reader. ~~The~~Fig. 3 shows the results of ~~osteoclasts formation by~~examining the abilities of ST2 cells ~~with a~~to support osteoclast formation, wherein the passage number of ~~about~~said cells were around 10's or around 40's (after ~~purchasing the cells~~purchased from ~~RIKEN CELL BANK are shown in Figure 3.~~Riken Cell Bank). ~~The~~From these results ~~indicate~~, it was revealed that ~~the~~ ST2 cells ~~with~~of a high~~er~~ passage number ~~exhibit more potent~~had a high ability to support osteoclast~~s~~- formation.

~~(2) Time course change of expression of the protein of the present invention on membrane of osteoblastic stromal cells in a culture system which include active-form vitamin D_3 and dexamethasone and time course change~~

(2) Changes with the Passage of Time in Expression of ~~osteoclasts formation~~the Protein of the Present Invention on the Menbrane of Osteoblast Like Stromal Cells Cultured in ~~the co-culture system~~ the Presence of the Active-Form of Vitamin D_3 and Dexamethasone and Those in Osteoclast Formation in a Co-Culture System

~~In the same manner as in Example 4(3), osteoblastic~~Osteoblast-like stromal cell line, ST~~2~~2, was cultured ~~for 7 days~~ in the presence of the active-form of vitamin D_3 and dexamethasone for 7 days in the same manner as in Example 4-(3). ~~The~~ OCIF-binding ~~test~~experiment was ~~carried out~~conducted using ^{125}I-labeled OCIF (monomer type) ~~as~~ described in ~~the experiment in~~ Example 4-(1). ~~Non-specific~~Nonspecific binding was measured by competing the ^{125}I-labeled OCIF ~~binding to ST2 cells~~ with a 400-fold higher concentration of unlabeled OCIF in binding to ST2 cells. As a result, ~~it was confirmed that~~ the amount of specific binding of the ^{125}I-labeled OCIF ~~increase in accordance with increase in culture period in~~was increased, due to the ~~presence of~~ active-form of vitamin D_3 and dexamethasone, with an increase in culturing days. ~~Specifically~~That is, as shown in Figures 4 and 5, the protein of the present invention was expressed on the cell surface of ST2 cells ~~by~~due to the active-form of vitamin D_3 ~~in accordance~~ with an increase in culture~~ing period~~days, and ~~the~~its expression reached ~~a~~ maximum on the fourth day of culture. On the other hand, osteoclast-like cells ~~are~~were formed ~~by coculturing~~after co-culture of mouse spleen cells and ST2 cells in the presence of the active-form of vitamin D_3~~-3~~. TRAP (a marker enzyme ~~of~~for osteoclasts)~~—~~positive mononuclear ~~pre~~-osteoclast-like cells ~~are~~were formed on the third or fourth day of ~~the~~ culture~~. More~~, and further, differentiated and matured TRAP-positive multinuclear cells ~~are~~were formed on the fifth ~~to~~or sixth day of ~~the~~ culture. ~~A good correlation between~~It was found that change with the passage of time ~~course of the~~ in expression of the protein of the present invention and ~~osteoclasts~~ in osteoclast formation ~~was thus demonstrated~~corresponded well with each other.

~~(3) Inhibition of osteoclasts formation by OCIF treatment for different period during the co-culture~~

(3) Effect of Inhibiting Osteoclast Formation When OCIF Was Treated Only During a Restricted Period of Co-Culture

To ~~make it clear~~further clarify that the protein of the present invention ~~is~~was a factor involved in ~~the~~osteoclast ~~osteoclasts~~ formation, ~~the~~ cells cultured during various periods (two days each, except for the fifth day) were treated with 100 ~~mg~~ng/ml of OCIF ~~for different culture periods during the six~~ in the above 6-day co-culture ~~period~~ described in ~~the above-mentioned~~ Example ~~5(2) (two consecutive days in the six-day period, except for the 5th day for which a one-day period was applied~~(2). As a result~~, as~~ shown in ~~Figure~~Fig. 6, in the case where OCIF ~~treatment at~~was added during the 48-th to 96~~-hours after start of~~th hr (as counted from the beginning of culture~~at which expression of~~), when the protein of the present invention was expressed at highest level on ST2 cells ~~is maximal was found to be most effective for inhibiting~~, osteoclast formation ~~of osteoclasts~~was inhibited most effectively. ~~Specifically~~That is, it was ~~confirmed~~revealed that OCIF ~~controls~~inhibited osteoclast formation by binding to ST2 cells via the protein of the present invention.

~~Based on the results of~~From the above ~~experiments~~results, it became clear that the protein of the present invention was ~~confirmed to be~~ induced on ~~cell membrane of osteoblastic~~the membranes of osteoblast-like stromal cells ~~by~~with the active-form of vitamin D_3 and dexamethasone, and ~~to exhibit a~~had the biological activity ~~to support or accelerate~~(effect) of a factor which supports and promotes differentiation ~~or~~and maturation of osteoclasts.

~~<~~[Example 6~~>~~]

Crosslinking ~~test for~~Experiment of ^{125}I-labeled OCIF ~~and~~to the ~~p~~Protein of the ~~p~~Present ~~i~~Invention

To further identify the presence of the protein of the present invention ~~more clearly~~, ^{125}I-labeled OCIF was allowed to crosslink with the protein of the present invention ~~was crosslinked with ^{125}I-labeled OCIF~~. ~~Mouse osteoblastic stromal~~As in Example 4-(3), mouse osteoblast like cell line, ST~~2~~2, was cultured ~~for four days~~ in the presence or absence of the active-form of vitamin D_3 and dexamethasone ~~in the same manner as described in Example~~for 4~~(3)~~ days. After ~~washing~~ the cells were washed with 1 ml of phosphate buffered saline solution, 200 ~~μ~~μl of medium for binding

~~test~~experiment (~~α~~α-MEM ~~to which~~medium containing 0.2% bovine serum albumin, 20 mM Hepes buffer, 0.2% NaN_{3}~~3~~ and 100 ~~μ~~μg/ml heparin ~~were added)~~, futher containing 25 ng/ml of the above ^{125}I-labeled OCIF (monomer type) or 40 ng/ml of ^{125}I-labeled OCIF-CDD1 ~~which~~was added. ^{125}I-labeled OCIF-CDD1 was obtained by expressing the protein ~~of Sequence~~described as SEQ ID ~~No.~~NO: 76 ~~(~~in WO 96/26217~~)~~ ~~in~~with animal cells~~, was added. The~~ and labeling in accordance with the above ~~mentioned culture~~ method. Furthermore, the medium for the binding ~~test was further supplemented with~~experiment, containing a 400-fold higher concentration of OCIF ~~and~~, was added to the other ~~wells~~well and was subjected to ~~assess non-specific~~an experiment for nonspecific binding. After ~~culturing for one hour~~the cells were cultured in a CO_2 incubator for 1 hour, ~~each well was~~they were washed three times with 1 ml of phosphate buffered saline solution containing 100 ~~μ~~μg/ml of heparin. ~~Five~~Then, ~~hundred~~500 ~~μ~~μl of phosphate buffered saline ~~containing~~solution in which 100 ~~μ~~μg/ml of crosslinking agent~~,~~ DSS (Disuccinimidyl suberate, Pierce Co.~~),~~, Ltd.) was dissolved was added ~~to each well~~thereto, and ~~the plate was kept~~allowed to react at 0°C for 10 minutes ~~at 0°C~~. ~~The~~After the cells in these wells were washed twice with 1 ml of phosphate buffered saline ~~at~~solution cooled to 0°C. ~~One~~, ~~hundred~~100 ~~μ~~μl of 20 mM Hepes buffer containing 1% Triton X-100, ~~10 μM pepstatin, 10 μM leupeptin,~~ 2 mM PMSF (phenylmethylsulfonyl fluoride), 10 μM pepstatin, 10 μM leupeptin, 10 ~~μ~~μM antipain~~,~~ and 2 mM EDTA~~,~~ was ~~then~~ added to each well. ~~The plate was allowed~~, and left to stand ~~for 30 minutes~~ at room temperature ~~to dissolve~~for 30 minute so as to lyse the cells. ~~Fifteen~~After 15 ~~μ~~μl of these samples were treated with SDS under non-reducing conditions ~~according to conventional method and subjected to~~in accordance with a commonly used method, they were run on a SDS-polyacrylamide ~~gel~~ electrophoresis ~~(4-~~gel (with a gradient of 4 to 20% polyacrylamide ~~gradient, manufactured by~~ Daiichi ~~Chemical~~Pure Chemicals Co., Ltd.). After electrophoresis, the gels ~~were~~was dried and exposed to ~~BioMax~~BIOMAX® MS film (~~manufactured by~~ Kodak Co., Ltd.) ~~for 24 hours at -80°C~~ using ~~BioMax~~BIOMAX® MS ~~intensifying screens (manufactured by~~ amplifying screen (Kodak~~)~~ Co., Ltd.) at -80°C for 24 hours. ~~After exposure, the film was~~The exposed films were developed ~~by~~in ~~conventional~~accordance with a commonly used method. ~~A band of crosslinking product with~~When the ^{125}I-labeled OCIF (monomer

type, 60 kDa) was used, a crosslinked protein having a molecular weight of about 90,000- to 110,000 was detected. On the other hand, when the ^{125}I-labeled OCIF ~~(monomer type, 60 kDa) was used. When the ^{125}I-labeled OCIF~~-CDD1 (31 kDa) was used, a ~~band of crosslinking product~~crosslinked protein of about 70- to 80 kDa (78 kDa on average~~, 78 kDa~~) was detected as shown in ~~Figure~~Fig. 7.

~~<~~[Example 7~~>~~]

Scatchard Plot Analysis of the ~~p~~Protein of the ~~p~~Present ~~i~~Invention ~~e~~Expressed on ST~~-cells by Scatchard Plot~~2 Cells

~~The above-mentioned ^{125}I-labeled OCIF (monomer type) was added to a concentration of 1,000 pM to the culture medium~~Medium for the binding ~~test~~experiment (e~~α~~-MEM medium containing 0.2% bovine serum albumin, 20 mM Hepes buffer~~,~~ and 0.2% NaN_3) ~~and the culture medium~~further containing 1,000 pM of the above ^{125}I-labeled OCIF (monomer type) was ~~serially~~prepared and diluted stepwise at a dilution rate ~~to~~of 1/2 with the ~~culture-~~medium ~~not containing ^{125}I-labeled OCIF~~for the binding experiment. ~~Solutions~~Furthermore, another medium for ~~measuring~~determining ~~non-specifie~~nonspecific binding ~~were~~was prepared by ~~further-~~adding a 400-fold higher concentration of unlabeled monomer~~-form~~ type OCIF to ~~these solutions~~the above medium. ~~Two hundred μ~~200 μl of these prepared solutions were added to ~~the above-mentioned~~ wells ~~with~~of the above ST2 cells (about 10^{th} passage), cultured for 4 days ~~(passage number, about 10)~~ in the presence of 10^{-8} M of the active-form of vitamin D_3 (~~C~~calcitriol) and 10^{-7} M dexamethasone, ~~to assess~~and binding of the ^{125}I-labeled OCIF was tested in the same ~~method~~manner as ~~described~~ in Example 4-(3). The obtained results were ~~subjected to~~ Scatchard ~~Plot analysis to determine the~~-plotted in accordance with a common method, and dissociation constants of OCIF and the OCIF- binding protein, and the number ~~(site) of OCIF~~ of the OCIF binding protein (site) per ~~a~~one ST2 cell were determined. As a result, the dissociation constants of OCIF and the protein of the present invention ~~was found to be~~were 280 pM, and the number of the ~~site of~~ OCIF-binding protein (site) per ~~a~~one ST2 cell was ~~approximately~~about 33,000/cell. ~~Based on the finding in Example 5(1) that osteoclasts formation supported by the~~Furthermore, a cultured ST2 cells with a passage ~~number about~~ number of around 40 ~~was more extensive~~'s had higher ability to support osteoclast formation than that with a passage number

~~about 10, the number (the site)~~of around 10's as shown in Example 5-(1), so that the number of sites of the protein of the present invention expressed on the ST2 cell with a passage number ~~about~~of around 40's was ~~assessed~~measured. ~~The~~As a result, the number ~~(~~of site~~)~~ was 58,000/cell ~~and~~which, was clearly ~~larger~~greater than that on the ST2 cells with a passage number ~~about 10, indicating~~of around 10's. It was revealed that the amount of the expression of the protein of the present invention ~~expressed on~~associated with the degree of the ability of ST2 ~~cells is related to their potency~~cell to support osteoclasts~~-~~ formation. The~~is results~~ finding indicate~~d~~s that the protein of the present invention is a factor ~~that~~ to support~~s or induces~~ and promote differentiation ~~or~~and maturation of osteoclasts.

~~<~~[Example 8~~>~~]

Cloning of ~~OBMcDNA~~OBM cDNA

(1) Extraction of RNA from ~~m~~Mouse ST2 ~~c~~Cells~~s~~

Mouse ~~osteoblastic~~osteoblast-like stromal cell line, ST~~2~~2, (~~RIKEN CELL BANK~~Riken Cell Bank, RCB0224) was ~~cultured in α~~with α-MEM medium (Gibco BRL Co., Ltd.) containing 10% ~~fetal~~ bovine fetal serum. ~~ST2 cells~~After cultured ~~to~~cells become confluent in ~~a~~ 225 -cm^2 T- flask~~s~~ for adherent cell culture, ST2 cells were treated with trypsin ~~to harvest the cells~~, stripped from the T- flask~~. The cells were~~, washed, and transferred to five 225 -cm^2 T- flasks. ~~Sixty~~After adding 60 ml of ~~α~~α-MEM medium containing 10^{-8} M of the active-form of vitamin D_3 (~~C~~calcitriol, Wako Pure Chemicals ~~Co.~~Industries, Ltd.), 10^{-7} M dexamethasone~~,~~ and 10% ~~fetal~~ bovine fetal serum ~~was added to each flask and~~thereto, the cells were cultured ~~for 5 days~~ in a CO_2 incubator for 5 days. Total RNA was extracted from the cultured ST2 cells using ISOGEN (Wako Pure Chemicals ~~Co.~~Industries, Ltd.). Poly A^+ RNA was prepared from about 600 ~~μ~~μg of the total RNA using an Oligo (dT)~~-~~-cellulose column (5'-3' Prime Co., Ltd.). About 8 ~~μ~~μg of ~~P~~poly A^+ RNA was obtained.

(2) Construction of ~~e~~Expression ~~l~~Library

Double- strand~~ed~~ cDNA~~s~~ ~~was~~ were synthesized from 2 ~~μ~~μg of ~~polyA~~the poly A^+ RNA obtained in Example 8-(1) ~~using a~~with Great Lengths cDNA Synthesis kit (Clontech Co., Ltd.) ~~according to the instruction~~ in ~~the~~accordance with a manual thereof. ~~Specifically~~More specifically, 2 ~~μ~~μg of ~~polyA~~the poly A^+ RNA and an Oligo (dT) $_{25}$ (dN)

primer were mixed ~~and~~together, distilled water was added ~~to the mixture to make~~thereto so that the final volume ~~to~~was 6.25 ~~μ~~μl. ~~After incubation~~, and the mixture was incubated at 70°C for ~~about~~ 3 minutes ~~at 70°C~~, ~~the mixture was~~and then cooled ~~on~~in ice for 2 minutes. ~~To the mixture were added~~Then, 2.2 ~~μ~~μl of distilled water, 2.5 ~~μ~~μl of 5X First-strand buffer, 0.25 ~~μ~~μl of 100 mM DTT (dithiothreitol), 0.5 ~~μ~~μl of PRIME RNase ~~i~~Inhibitor (1 U/ml) (5'-3' Prime Co., Ltd.), 0.5 ~~μ~~μl of [~~α~~α-^{32}P] dCTP (Amersham Co., ~~3000~~Ltd., 3,000 Ci/mmol~~)~~, 2 μCi/μl) which was diluted ~~5-fold with distilled water to make 2 μCi/μl~~to be one fifth concentration, 0.65 ~~μ~~μl of dNTP (20 mM each)~~,~~ and 1.25 ~~μ~~μl (250 units) of MMLV (RNaseH$^-$) reverse transcriptase were added thereto, respectively. ~~The mixture~~Thus obtained solution was incubated at 42°C for 90 minutes ~~at 42°C~~. Then, ~~followed by the further addition of~~ 62.25 ~~μ~~μl of distilled water, 20 ~~μ~~μl of 5X second-strand buffer, 0.75 ~~μ~~μl of dNTP (20 mM each)~~,~~ and 5 ~~μ~~μl of Second-strand enzyme cocktail. ~~The resulting mixture~~ were added thereto, respectively. Thus obtained solution was ~~maintained~~incubated at 16°C for ~~two~~2 hours. ~~Then,~~ 7.5 units of T4 DNA polymerase was added ~~to this reaction mixture. After incubation~~thereto, and further incubated at 16°C for another 30 minutes. Thereafter, ~~the reaction was terminated by the addition of~~ 5 ~~μ~~μl of 0.2 M EDTA. ~~After~~ was added to terminate the reaction, and after a phenol-chloroform treatment, ~~the product was precipitated with e~~thanol precipitation was carried out. An EcoRI-SalI-NotI linker (Clontech Co., Ltd.) was ~~attached~~added to ~~the~~an ends of the ~~resultant~~ double- strand~~ed~~ cDNA. ~~Then, the ends were phospholylated~~ and ~~the product was applied on~~then phosphorylated at its end. Using a column for size fractionation ~~column to obtain cDNA with a length more~~, cDNAs of not smaller than 500 bp. ~~DNA was~~ were separated, and ethanol-precipitated. The precipitated ~~with ethanol, dissolved~~DNAs were reconstituted in water and ~~ligated~~inserted ~~to~~into pcDL-SR ~~α~~-α296 (Molecular and Cellular Biology, Vol. 8, pp. 466- to 472, 1988) ~~which had been cut~~(Takara Shuzo Co., Ltd.) previously cleaved with a restriction enzyme, EcoRI ~~(Takara Shuzo Co.)~~, and subsequently treated with CIAP (calf intestine alkaline phosphatase, Takara Shuzo Co., Ltd.).

(3) Screening of eExpression lLibrary by means of in Which the bBinding to OCIF Was Used as an Index

An escherichia*E. coli* strail, XL2 Blue MRF' (Toyobo Co., Ltd.), was transformed usingwith the DNA producedobtained in Example 8-(2), and culturedallowed to grow on a L-Carbenisilin agar Carbenicillin Agar Medium (1% trypton, 0.5% yeast extract, 1% NaCl, 60 μμg/ml carbenisilin,carbenicillin and 1.5% agar) prepared in a 24-well plastic plates,plate for cell culture so that the cells was grown to produce about 100 colonies per well. TransformantsThe transformants in each well were suspended in 3 ml of Terrific Broth ampicillin culture medium (1.2% trypton, 2.4% yeast extract, 0.4% glycerol, 0.017 M KH_2PO_4, 0.072 M K_2HPO_4, 100 μμg/ml ampicillin), and cultured with shaking at 37°C overnight with shaking. Cells wereThe *E. coli* was collected by centrifugation to prepare, and plasmid DNA using a QIAwellDNAs were prepared therefrom with QIAWELL® kit (QIAGEN Co., Ltd.). The DNA concentrationcontent was determined by measuringdetecting absorbance at 260 nm. DNA, and the DNAs was concentrated by precipitating with ethanol precipitation and dissolved in distilled water to aso that the concentration ofwas 200 ng/μμl. Five hundredThus, 500 DNA pools, each of which was obtainedderived from about 100 colonies were prepared and were used for transfection intoof COS-7 cells (RIKEN CELL BANKRiken Cell Bank, RCB0539). COS- 7 cells were seeded into DMEM containing 10% fetal bovine serum in each well ofa 24-well plates at a cell density ofplate so as to achieve 8 X 10^4 cells/well and cultured overnight at 37°C in a CO_2 incubator at 37°C overnight by use of DMEM medium containing 10% bovine fetal serum. NextOn the following day, the culture medium was removed, and the cells were then washed with serum-free DMEM culture medium. The above describedIn accordance with a protocol attached to lipofectamine (Gibco Co., Ltd.) which was a reagent for transfection, the plasmid DNA which was previously diluted with an OPTI-MEM culture® medium (Gibco BRL Co., Ltd.) and lipofectamine were mixed with Lipofectamine (a transfection reagenttogether, manufactured by Gibco BRL Co.) according to the protocol supplied with Lipofectamine. Afterand after 15-minute minutesinculation, the mixture was added to the cells in each well. The amounts of LipofectamineDNA and DNAlipofectamine used were, respectively, 1 μμg and 4 μμl per well, respectively. After 5-hour hoursincubation, the culture medium was removed, and

1 ml of DMEM ~~culture~~ medium (Gibco BRL Co., Ltd.) containing 10% ~~fetal~~ bovine fetal serum was added ~~to each well. The plates were incubated for 2-3 days at 37°C~~and cultured in a CO_2 incubator (5% CO_2) at 37°C for 2 to 3 days. The COS-7 cells ~~transfected~~obtained after transfection and ~~cultured~~subsequent culture for 2- to 3 days ~~in this manner~~ were washed with ~~a~~ serum-free DMEM ~~culture~~ medium. Then, 200 ~~μ~~μl of ~~a~~ ~~culture~~ medium for ~~the~~ binding ~~assay~~experiment (serum-free DMEM ~~culture~~ medium containing 0.2% ~~calf~~bovine serum albumin, 20 mM Hepes buffer, 0.1 mg/ml heparin~~,~~ and 0.02% NaN_3) ~~with~~further containing 20 ng/ml of ^{125}I-labeled OCIF was added thereto ~~was added to each well. After culturing for one hour at 37°C~~Cells were cultured in a CO_2 incubator (5% CO_2)~~, the cells were~~ at 37°C for 1 hour and washed twice with 500 ~~μ~~μl of ~~a~~ phosphate buffered saline solution containing 0.1 mg/ml heparin. After washing, 500 ~~μ~~μl of 0.1 N NaOH solution was added ~~to each well. The plates were allowed~~thereto, and then left to stand ~~for 10 minutes~~ at room temperature for 10 minutes so as to l~~y~~yse the cells. The amount of ^{125}I in each well was measured ~~using~~with a gamma counter (Packard Co., Ltd.). ~~One DNA pool containing cDNA encoding the protein which specifically binds to OCIF was found by~~After screening ~~a total of~~the 500 pools~~. The~~ in total, a DNA pool containing ~~the~~a cDNA encoding a protein that could specifically bind OCIF was ~~further divided~~isolated. Furthermore, the DNA pools containing the cDNA of the present invention were subfractionated, and then employed to repeat the above-~~described~~ transfection and screening ~~operations were repeated to isolate the~~. Thereafter, a cDNA ~~which encodes the~~encoding a protein which ~~binds~~could ~~to~~bind OCIF was isolated. ~~The~~A plasmid containing thi~~s~~e cDNA was ~~named~~referenced pOBM291. ~~The Escherichia~~*E. coli* containing thi~~s~~e plasmid was deposited ~~with~~at ~~T~~the National Institute of Bioscience and Human ~~-~~Technology, Agency of Industrial Science and Technology~~,~~ ~~Biotechnology Laboratory,~~ as pOBM291 ~~on May 23, 1997 under~~with the deposition ~~No.~~number of FERM BP-~~5953. The methods of~~ 5953 on May 23, 1997. Methods for ^{125}I-labeling of OCIF ~~with~~ ^{125}~~I~~ and ~~quantitative analysis of~~ determining (the concentration of) ^{125}I-labeled OCIF by ELISA are ~~shown~~as ~~below~~follows. ~~Labeling of~~ OCIF was ^{125}I-labeled in accordance with ^{125}~~I was carried out according to the~~ Iodogen method. ~~Twenty~~20 ~~μ~~μl of ~~25~~2.5 mg/ml Iodogen-chloroform solution was ~~added~~transferred to a 1.5 ml Eppendorf tube, and chloroform was evaporated ~~by heating~~ at 40°C~~,~~ so as to

prepare an Iodogen-coated tube. ~~The~~After the tube was washed three times with 400 ~~μ~~μl of 0.5 M sodium phosphate buffer (Na-Pi, pH 7.0), ~~and 5~~0.5 ~~μ~~μl of 0.5 M Na-Pi ~~(~~with a pH of 7.0~~)~~ was added thereto. Immediately after ~~the addition of~~ 1.3 ~~μ~~μl (18.5 MBq) of Na-^{125}I solution (~~NEZ-033H20, Amersham Co.~~), Ltd., NEZ-033H20) was added thereto, 10 ~~μ~~μl of 1 mg/ml ~~rOCIF~~OCIF solution (monomer type or dimmer type) was added ~~to the tube~~. ~~After mixing the contents~~The resulting solution was agitated with a vo~~l~~rtex mixer~~, the tube was allowed~~ and then left to stand at room temperature for 30 seconds. The~~is~~ solution ~~in the tube~~ was transferred to a tube ~~to~~in which ~~80 μl of~~ 10 mg/ml potassium iodide, 80 μl of 0.5 M Na-Pi solution (pH 7.0) and 5 ~~μ~~μl of ~~a~~ phosphate buffered saline solution containing 5% bovine serum albumin (BSA-PBS) ~~were~~was previously added and then agitated. ~~After stirring, the mixture~~This solution was applied to a spin column (1 ml, G-25 fine, ~~manufactured by~~ Pharmacia Co., Ltd.) equilibrated with BSA-PBS~~,~~ and ~~the column was centrifuging for 5 minutes~~centrifuged at ~~2000~~2,000 rpm for 5 minutes. ~~Four~~After ~~hundred~~400 ~~μ~~μl of BSA-PBS was added to ~~the fraction eluted~~an eluate from the column~~. After stirring~~ and mixed, 2 ~~μ~~μl ~~of an aliquot of this solution~~ was ~~sampled to measure the~~subfractionated and its radioactivity ~~by~~was measured with a gamma counter. The radiochemical purity of ~~the ^{125}I labeled OCIF solution~~ thus prepared ^{125}I-labeled OCIF solution was determined by measuring the radioactivity of a fraction precipitated ~~by~~with 10% TCA. ~~The~~Furthermore, the biological activity as OCIF of the ^{125}I-labeled OCIF solution was ~~measured according to the~~determined in accordance with a method ~~of~~described in WO 96/26217. ~~The~~Moreover, the concentration of ~~the~~ ^{125}I-labeled OCIF was ~~determined~~measured by ~~the~~ ELISA ~~as follows~~in the following manner. ~~Specifically~~That is, 100 ~~μ~~μl of 50 mM $NaHCO_3$ (pH 9.6) in which ~~the~~2 μg/ml of anti-OCIF rabbit polyclonal antibody described in WO 96/26217 was dissolved ~~to a concentration of 2 μg/ml~~ was added to each well of a 96-well ~~immuno-plate~~immunoplate (~~MaxiSorp™, a product of~~ Nunc Co.~~). The plate was allowed~~, Ltd., MaxiSorp) and left to stand ~~over night~~ at 4°C overnight. After ~~removing the~~this solution ~~by~~was ~~suction~~removed, ~~300~~200 ~~μ~~μl of ~~Block Ace™~~a combined solution of of BLOCKACE (Snow Brand Milk Products Co., Ltd.) ~~/~~and phosphate buffered saline solution (mixing ratio = 25~~/~~:75~~)~~: ~~(B-PBS~~BPB) was added to each well~~. The plate was~~ and then ~~allowed~~left to stand ~~for two hours~~ at room temperature for 2 hours. After ~~removing the~~this solution

~~by~~was ~~suction~~removed, ~~the~~ each well~~s were~~ was washed three times with phosphate buffered saline solution (P-PBS) containing 0.01% Polysorbate ~~80 (P-PBS).~~80. ~~Next~~Thereafter, 100 ~~μ~~μl of B-PBS containing a ^{125}I-labeled OCIF or standard OCIF was added ~~to each well. The plate was then allowed~~thereto and left to stand ~~for two hours~~ at room temperature for 2 hours. After ~~removing the~~this solution ~~by~~was ~~suction~~removed, each well was washed six times with 200 ~~μ~~μl of P-PBS. ~~One hundred μl of~~Then, a peroxidase-labeled ~~rabbit~~ anti-OCIF rabbit polyclonal antibody was diluted with B-PBS and 100 μl of the diluted solution was added to each well~~. The plate was allowed~~, and then left to stand ~~for two hours~~ at room temperature for 2 hours. After ~~removing the~~this solution ~~by~~was ~~suction~~removed, ~~the~~ each well~~s were~~ was washed six times with 200 ~~μ~~μl of P-PBS. Then, 100 ~~μ~~μl of TMB solution (TMB Soluble Reagent, High Sensitivity, Scytek Co., Ltd.) was added to each well~~. After incubating the plate~~ and then left to stand at room temperature for 2- to 3 minutes. Thereafter, 100 ~~μ~~μl of ~~stopping solution~~ (~~Stopping Reagent~~, (Scytek Co., Ltd.) was added ~~to~~thereto. The absorbance of each well~~. Absorbance~~ at 450 nm ~~of each well~~ was measured ~~using~~with a microplate reader. The concentration of the ^{125}I-labeled OCIF was determined ~~based on~~from a calibration curve ~~drawn~~made using ~~the~~ standard ~~preparation of~~ OCIF.

(4) Determination of the ~~n~~Nucleotide ~~s~~Sequence of ~~the~~ cDNA ~~encoding~~Which Eccodes the ~~entire amino acid sequence~~Full Length Amino Acid Sequence of OBM

The nucleotide sequence of ~~the~~ OBM cDNA obtained in ~~the~~ Example 8-(3) was determined ~~using a~~with Taq ~~DyeDeoxy~~Dye Deoxy Terminator Cycle Sequencing kit (~~a product of~~ Perkin Elmer Co., Ltd.). ~~Specifically~~That is, using pOBM291 as a template, the nucleotide sequence of the inserted fragment was directly determined ~~using pOBM291 as a template~~. ~~Fragments with a length of~~Furthermore, about 1.0 kb and about 0.7 kb ~~which were~~fragments obtained by ~~digesting~~cleaving pOBM291 with a restriction enzyme, EcoRI, were inserted into ~~the~~ EcoRI site of plasmid pUC19 (Takara Shuzo Co., Ltd.) and sequenced, respectively. ~~The nucleotide sequences of these fragments were also determined. The following primers were used:~~ A primer SRR2 ~~which was used to determine nucleotide sequences of~~for sequencing the DNA fragments inserted ~~into~~in pcDL-SR ~~ex~~α296, primers M13PrimerM3 and M13PrimerRV (~~both manufactured by~~ Takara Shuzo Co., Ltd.) ~~which were used to determine~~for sequencing the ~~nucleotide~~

~~sequences of~~ DNA fragments inserted ~~into~~in the plasmid pUC19, and ~~synthesized~~a synthetic primer OBM #8 designed based on the nucleotide sequence of ~~OBMcDNA~~OBM cDNA were used. ~~Sequences~~ The sequences of these primers are shown as ~~the Sequence~~SEQ ID ~~No.~~NOs: 3 to ~~No. 6 in the sequence table.~~6.

~~In addition~~Furthermore, the determined nucleotide sequence of ~~OBMcDNA~~OBM ~~determined~~cDNA is shown as ~~Sequence~~SEQ ID ~~No.~~NO: ~~2~~2, and the deduced an amino acid sequence ~~determined therefrom~~ is shown as ~~the Sequence~~SEQ ID ~~No.~~NO: 1.

~~<~~[Example 9~~>~~]

Expression of the ~~p~~Protein ~~e~~Encoded by the cDNA of the ~~p~~Present ~~i~~Invention

~~Plasmid pOBM291 was~~ COS-7 cells were transfected ~~into COS-7 cells~~with plasmid pOBM291 with lipofectamine in each well of a 6-well plate ~~using Lipofectamine~~, and ~~the transfected COS-7 cells~~ were cultured ~~for two days~~ in DMEM medium containing 10% ~~fetal~~ bovine fetal serum for 2 days. The medium was replaced with ~~a~~ cysteine-/methionine-free DMEM (~~Dainippon Seiyaku Co~~DAINIPPON PHARMACEUTICAL CO., ~~Ltd~~LTD.) ~~(800 µl/well) containing~~ in which 5% dialyzed ~~fetal~~ bovine fetal serum. ~~The~~ (800 µl/well) was added, and the cells were cultured for another 15 minutes. Then, ~~followed by the addition of~~ 14 ~~µ~~µl of Express Protein Labeling Mix (NEN CO., LTD., 10 mCi/ml~~, manufactured by NEN Co.~~) was added thereto. After ~~culturing~~the cells were cultured for ~~four~~4 hours, 200 ~~µ~~µl of DMEM ~~including~~medium containing 10% ~~fetal~~ bovine fetal serum was added, and the cells were cultured for 1 hour. After ~~one hour culturing~~, the cells were washed twice with PBS. ~~Then~~, 0.5 ml of ~~a~~ TSA buffer (10 mM Tris-HCl (pH 8.0) containing 0.14 M NaCl~~,~~ and 0.025% NaN_3)~~,~~ containing 1% ~~TritonX~~Triton X-100, 1% bovine hemoglobin, 10 ~~µ~~µg/ml leupeptin, 0.2 TIU/ml aprotinin~~,~~ and 1 mM PMSF~~,~~ was added, and the ~~mixture was allowed~~cells were left to stand ~~for one hour~~ on ice for 1 hour. ~~Cells~~After the cells were ~~disrupted~~crushed by pipetting, centrifugation was carried out at 3,000 xg at 4°C and ~~centrifuged at 3000 x g~~ for 10 minutes ~~at~~so ~~4°C~~as to obtain a supernatant. To 100 µl of this supernatant, 200 ~~µ~~µl of dilution buffer (TSA buffer containing 0.1% ~~TritonX~~Triton X-100, 0.1% bovine hemoglobin, 10 ~~µ~~µg/ml leupeptin, 0.2 TIU/ml aprotinin~~,~~ and 1 mM PMSF) was added ~~to 100 µl of this~~, and the resulting supernatant. ~~The mixture~~ was shaken ~~for one hour at 4°C~~together with ~~p~~Protein A Sepharose® (50 ~~µ~~µl). ~~The resultant~~

~~mixture was~~ at 4°C for 1 hour, and then centrifuged at ~~1500 x~~4°C and 1,500 X g for ~~one~~1 minute ~~at~~so ~~4°C~~as to collect a supernatant. Thereby, ~~and thereby~~a fraction~~(s) which is non-specifically adsorbed~~ nonspecifically binding to the Protein A Sepharose® was removed. OCIF (1 ~~μ~~μg) was added to this supernatant, and the ~~mixture~~obtained supernatant was shaken at 4°C for ~~one~~1 hour ~~to achieve the binding of~~so that OBM bound OCIF ~~to OBM~~. ~~Anti~~Then, an anti-OCIF polyclonal antibody (50 ~~μ~~μg) was added, and the ~~mixture~~solution was shaken at 4°C for ~~one~~1 hour ~~at 4°C~~. Then, Protein A Sepharose® (10 ~~μ~~μl) was further added, and the ~~mixture~~solution was further shaken at 4°C for ~~an~~another ~~additional~~1 hour ~~at 4°C, followed by centrifuge~~. The solution was centrifuged at 1,500 xg at ~~1500 x g~~4°C for 1 minute ~~at 4°C to collect~~ and the precipitated fraction was collected. The precipitate. ~~The precipitate~~ resulting from centrifugation at 1,500 xg at 4°C for 1 was washed twice with the dilution buffer, twice with a~~the~~ dilution buffer without bovine hemoglobin ~~free dilution buffer~~, once with TSA buffer, and once with 50 mM Tris-HCl (pH 6.5). After washing, SDS buffer (0.125 M Tris-HCl, 4% dodecyl sodium ~~dodecylsulfate~~sulfate, 20% glycerol, 0.002% ~~B~~bromophenol ~~B~~blue, pH 6.8) containing 10% ~~β~~-β mercaptoethanol was added to the precipitate. The ~~mixture~~precipitate was heated at 100°C for 5 minutes ~~at 100°C~~ and subjected to SDS-PAGE (12.5% polyacrylamide gel, Daiichi ~~Chemical~~Pure Chemicals Co., Ltd.). ~~The~~After the gel was fixed ~~according to~~in accordance with a ~~conventional~~commonly used method. ~~Isotope~~, signals of isotope were amplified ~~using~~with Amplify™ (Amersham Co., Ltd.), and the ~~sample~~fixed gel was exposed to ~~Bio-Max~~BioMax® MR film (~~KODAK~~Kodak Co., Ltd.) at –80°C. The results are shown in ~~Figure 8, which indicates that~~Fig. 8. The molecular weight of the protein encoded by the cDNA of the present invention ~~has a molecular weight of~~was found to be about 40,000.

~~<~~[Example 10~~>~~]

Binding of the ~~p~~Protein ~~e~~Encoded by the cDNA of the ~~p~~Present ~~i~~Invention to OCIF

~~Plasmid pOBM291 was~~COS-7 cells were transfected ~~into COS cells~~with plasmid pOBM291 with lipofectamine in ~~each well~~wells of a 24-well plate ~~using Lipofectamine. After culturing~~and cultured for 2- to 3 days. Then, the cells were washed with serum-free DMEM ~~culture~~ medium. –, and 200 ~~μ~~μl of ~~culture~~ medium for ~~the~~ binding ~~assay~~experiment (serum-free DMEM ~~culture~~ medium containing 0.2% ~~calf~~bovine serum,

albumin, 20 mM Hepes buffer, 0.1 mg/ml heparin~~,~~ and 0.2% NaN_3)~~, supplemented with~~ containing 20 ng/ml of ^{125}I-labeled OCIF~~,~~ was added ~~to the wells~~thereto. ~~To the other wells~~Furthermore, 200 ~~μ~~μl of ~~culture~~the medium for ~~the~~ binding ~~assay to which~~experiment containing 8 ~~μg~~μg/ml of unlabe~~l~~led OCIF ~~had been added,~~ in addition to 20 ng/ml of the ^{125}I-labeled OCIF~~, was added~~ was added to other wells. ~~After culturing for one hour at 37°C~~The cells were cultured in a CO_2 incubator (5% CO_2)~~, the cells were~~ at 37°C for 1 hour, and washed twice with 500 ~~μ~~μl of phosphate buffered saline solution containing 0.1 mg/ml ~~of~~ heparin~~. Then~~. After washing, 500 ~~μ~~μl of 0.1 N NaOH solution was added to each well, and then the ~~plate~~well was ~~allowed~~left to stand ~~for 10 minutes~~ at room temperature for 10 minutes so as to ~~dissolve~~lyse the cells. The amount of ^{125}I in each well was measured ~~by~~with a gamma counter. As a result, ~~as shown in Figure 9,~~it was confirmed that the ^{125}I-labeled OCIF ~~was found to bind~~bound only to ~~the~~ cells ~~in which~~transfected with the plasmid pOBM~~291 was transfected~~291 as shown in Fig. 9. ~~In addition~~Further, it was also confirmed that the binding was ~~confirmed to be conspicuously~~significantly inhibited by ~~the~~ addition of ~~(unlabeled) OCIF at~~ a 400-fold higher concentration of (unlabeled) OCIF. ~~These~~From these results ~~have demonstrated~~, it was revealed that ~~the~~ OBM, a protein ~~OBM~~ encoded by the cDNA ~~on~~of the plasmid pOBM~~291~~291 specifically ~~binds to~~bound OCIF on the surface of ~~the transfected~~ COS-7 cells.

~~<~~[Example 11~~>~~]

Crosslinking Experiment of ^{125}I-labeled OCIF ~~and~~to the ~~p~~Protein ~~e~~Encoded by the cDNA of the ~~p~~Present ~~i~~Invention

~~Crosslinking of ^{125}I-labeled monomer type OCIF and the protein encoded by the cDNA of the present invention was carried out~~In order to ~~investigate~~analyze the characteristics of the protein encoded by the cDNA of the present invention ~~in further detail~~more specifically, ^{125}I-labeled monomer type OCIF was allowed to crosslink with the protein encoded by the cDNA of the present invention. ~~After transfection of~~COS-7 cells were transfected with plasmid pOBM291 ~~into COS-7 cells according to~~in accordance with the method ~~used~~ described in ~~the~~ Example 8 ~~-~~(3), 200 ~~μ~~μl of ~~the culture~~ medium for ~~the~~ binding ~~assay, as described above,~~experiment containing the above ^{125}I-labeled OCIF (25 ng/ml) was added ~~to the wells~~thereto. ~~The~~Furthermore, ~~culture~~the

medium for the binding ~~assay to which unlabeled OCIF at~~experiment containing a 400-fold higher concentration ~~was added~~of unlabeled OCIF in addition to the ^{125}I-labeled OCIF was added to ~~the~~ other wells. ~~After culturing for one hour at 37°C~~The cells were cultured in a CO_2 incubator (5% CO_2)~~, the cells were~~ at 37°C for 1 hour and washed twice with 500 ~~μ~~μl of phosphate buffered saline solution containing 0.1 mg/ml of heparin. ~~Five hundred~~500 ~~μ~~μl of phosphate buffered saline solution containing 100 ~~μ~~μg/ml of ~~a~~ crosslinking agent~~,~~ DSS (~~d~~Disuccinimidyl suberate, ~~manufactured by~~ Pierce Co., Ltd.) was added to these cells, ~~followed by a reaction~~and the cells were allowed to react at 0°C for 10 minutes ~~at 0°C.~~. ~~The~~ After the reaction, the cells in these wells were washed twice with 1 ml of ~~cold~~ phosphate buffered saline ~~(~~solution cooled to 0°C~~)~~. ~~After the addition of~~ Then, 100 ~~μ~~μl of 20 mM Hepes buffer containing 1% Triton X-100 (Wako Pure ~~Chemicals Co.~~Chemical Industries, Ltd.), 2 mM PMSF (~~P~~phenylmethylsulfonyl fluoride, Sigma Co.~~), 10 μM Pepstatin (Wako Pure Chemicals Co.~~, Ltd.), 10 ~~μ~~μM ~~Leupeptin~~pepstatin (Wako Pure ~~Chemicals Co~~Chemical Industries, Ltd.), 10 μM leupeptin (Wako Pure Chemical Industries, Ltd.), 10 ~~μ~~μM antipain (Wako Pure ~~Chemicals Co.~~Chemical Industries, Ltd.) and 2 mM EDTA (Wako Pure Chemicals ~~Co.~~Industries, Ltd.) was added to these cells, and the wells were ~~allowed~~left to stand ~~for 30 minutes~~ at room temperature for 30 minute so as to ~~dissolve~~lise the cells. ~~Fifteen~~After 15 ~~μ~~μl ~~aliquots~~ of these samples were ~~heated in the presence of~~treated with SDS under non-reducing conditions ~~according to~~in accordance with a ~~conventional~~commonly used method ~~and~~, they were subjected to electrophoresis with gel for SDS-electrophoresis ~~using~~(gradient of 4- to 20% polyacrylamide ~~gradient gel (Daiichi Pure Chemical Co~~, DAIICHI PURE CHEMICALS CO., ~~Ltd~~LTD.). After ~~the~~ electrophoresis, the gel was dried and exposed ~~for 24 hours at -80°C~~ to ~~a~~ BioMax® MS film (Kodak Co., Ltd.) ~~using a~~with BioMax® MS ~~sensitization~~amplifying screen (Kodak Co., Ltd.) at -80°C for 24 hours. The exposed films ~~was~~were developed ~~according to~~in accordance with a ~~conventional~~commonly used method. As ~~a result, a band with a molecular weight of a range of 90, 000-110,000, shown in Figure 10, was detected by~~results of crosslinking of the ^{125}I-labeled monomer type OCIF ~~and~~with the protein encoded by the cDNA of the present invention, a band having a molecular weight of about 90,000 to 110,000 was detected as shown in Fig. 10.

~~<~~[Example 12~~>~~]

Northern ~~blotting~~ Blot a~~A~~nalysis

ST2 cells were cultured to become confluent in a 25 -cm^2 T flask, for ~~attached cell cultures were~~culturing adherent cells, and treated with trypsin ~~and~~. After being stripped from the T flask~~. After washing~~, the cells were washed and seeded ~~into~~in a 225 -cm^2 T flask ~~and cultured for 4 days in a CO_2 incubator with~~. 60 ml of ~~an α~~α-MEM ~~culture~~ medium containing 10^{-8} M of the active-form of vitamin $D_{3;3}$ 10^{-7} M dexamethasone~~,~~ and 10% bovine fetal ~~bovine~~ serum was added thereto, and the cells were cultured in a CO_2 incubator for 4 days. ~~Total~~Then, total RNA was extracted from the above-cultured ST2 cells ~~using~~with ISOGEN (Wako Pure Chemicals ~~Co.~~Industries, Ltd.). ~~The~~In addition, total RNA was ~~also~~ extracted ~~in the same manner~~ from ST2 cells ~~which were~~ cultured in the absence of the active-form of vitamin D_3 and dexamethasone~~.~~ ~~After~~ in accordance with the ~~addition of~~above method. To 20 μg (4.5 μl) of each total RNA sample, 2.0 ~~μ~~μl of 5X gel electrophoresis buffer ~~solution~~ (0.2 M ~~morpholino propane sulfonic~~morpholinopropanesulfonic acid~~,~~ (pH ~~7.0,~~7.0), 50 mM sodium acetate, 5 mM EDTA) ~~and~~, 3.5 ~~μ~~μl of formaldehyde~~,~~ and 10.0 ~~μ~~μl of formamide ~~to 20 μg (4.5 μl) of each of the total RNAs, the mixtures~~were added. The total RNA samples were incubated at 55°C for 15 minutes ~~at 55°C~~ and subjected to electrophoresis. ~~The gel~~Gels for electrophoresis ~~was prepared according to the formulation~~consisted of 1.0% agarose, 2.2 M ~~deionized~~ionized formaldehyde, 40 mM ~~morpholinopropane sulfonic~~morpholinopropanesulfonic acid (pH 7.0), 10 mM sodium acetate~~,~~ and 1 mM EDTA. ~~The~~Moreover, the electrophoresis was ~~carried out~~performed in ~~a~~ buffer ~~solution of~~comprising 40 mM ~~morpholino propane sulfonic~~morpholinopropanesulfonic acid~~,~~ (pH ~~7.0,~~ 7.0), 10 mM sodium acetate~~,~~ and 1 mM EDTA. After the electrophoresis, the RNA was transferred ~~onto~~to nylon membranes. About 1.0 kb D~~N~~FA fragments ~~was~~ were obtained by ~~digesting~~cleaving pOBM291 with a restriction enzyme, EcoRI~~.~~ ~~Hybridization was carried out using this DNA fragment~~, and labeled with ~~a Megaprime DNA labeling kit (Amersham Co.) and α~~α-^{32}~~p~~P-dCTP (Amersham Co.~~),~~, Ltd.) using MEGAPRIME DNA Labeling Kit (Amersham Co., Ltd.), and thus used as ~~a probe~~probes for hybridization. As a result, ~~as shown in Figure 11,~~ it was ~~confirmed~~revealed that ~~when ST2 cells were cultured in the presence of active-form vitamin D_3 and dexamethasone,~~

gene expression of the protein (OBM) encoded by the cDNA of the present invention ~~(OBM)~~was ~~is~~strongly induced ~~strongly~~in the ST2 cells cultured in the presence of the active-form of vitamin D_3 and dexamethasone.

~~<~~[Example 13~~>~~]

~~Osteoclasts formation supporting capability~~Ability of the ~~p~~Protein ~~e~~Encoded by the cDNA of the ~~present invention~~Present Invention to Support Osteoclast Formation

~~pOBM291 was transfected into COS cells according to~~ In accordance with the ~~same~~ method described in ~~the~~ Example 8~~(3).~~ (3), COS-7 cells were transfected with pOBM219. After ~~three~~3-day ~~days~~incubation, ~~trypsinized~~the cells were treated with trypsin and then centrifuged ~~by centrifugation~~-washed once with phosphate buffered saline solution. Then, ~~then~~the cells were fixed ~~with~~at room temperature for 5 minutes in suspension of PBS containing 1% paraformaldehyde ~~at room temperature for 5 minutes, followed by washing with PBS~~and then centrifuged-washed six times ~~by~~with ~~centrifugation~~PBS. ~~700 µl of 1 X 10^6 /ml mouse~~Mouse spleen cells and ~~350 µl of 4 X 10^4 /ml~~ ST2 cells ~~which~~ were ~~suspended in c~~prepared with α-MEM ~~culture~~ medium containing 10^{-8} M of the active-form of vitamin D_3, 10^{-7} M dexamethasone~~,~~ and 10% bovine fetal ~~bovine~~ serum~~, were~~ so that the cell concentration become 1 X 10^6 cells/ml or 4 X 10^4 cells/ml and then added to a 24-well plate~~.~~ in a volume of 700 µl and 350 µl, respectively. Furthermore, TC insert (Nunc Co., Ltd.) was set in each well. The ~~above-described~~ fixed COS cells (350 ~~µ~~µl) diluted ~~to various concentrations~~stepwise with the above~~-mentioned culture~~ medium and OCIF ~~solution~~ (50 ~~µ~~µl), were added to ~~the~~ TC inserts and cultured at 37°C for 6 days~~ at 37°C~~. As a result, it was ~~confirmed~~revealed that ~~the osteoclasts formation inhibitive~~an activity of OCIF ~~can be inhibited~~to inhibit osteoclast formation was suppressed by the protein encoded by the cDNA of the present invention.

~~<~~[Example 14~~>~~]

Expression of ~~s~~Secrete~~dory form~~-type OBM

(1) Construction of ~~a plasmid~~Plasmid for ~~the expression of secreted~~Expressing Secretory~~ form~~Type OBM Expression

A PCR reaction was carried out using OBM HF (~~Sequence Table, Sequence~~SEQ ID ~~No.~~NO: 7)~~ and~~/OBM XR (~~Sequence Table, Sequence~~SEQ ID ~~No.~~NO: 8) and

pOBM291 as primers and ~~pOBM291 as~~ a template, respectively. After ~~purification by~~the reaction product was purified through agarose gel electrophoresis, ~~the product was~~ it was digested with the restriction enzymes, HindIII and EcoRI, and ~~further~~then purified ~~by~~through agarose gel electrophoresis again. The purified fragment (0.6 kb), ~~Hind III~~HindIII/EcoR~~I~~V fragment (5.2 kb) of pSec TagA (Invitrogen Co.~~)~~, Ltd.) and EcoRI/PmaeCI fragment (0.32 kb) of OBM cDNA ~~were ligated using a a~~was subjected to ligation ~~kit ver~~using Ligation Kit Ver. 2 (Takara Shuzo Co., Ltd.), and subsequently *E.* ~~Escherichia~~ *coli* DH5-~~α~~α was transformed ~~using~~by the ~~reaction~~ligation product. Plasmids ~~were~~was purified ~~by means of alkali-SDS method~~ from the ~~resulting~~obtained ampicillin -resistant strains ~~and digested~~by alkaline-SDS method and then cleaved with restriction enzymes so as to select a plasmid ~~with fragments of a length of~~wherein 0.6 ~~Kb~~kb and 0.32 kb ~~being~~of fragments were inserted into pSec TagA. ~~Selected~~The selected plasmid was ~~identified as having a~~subjected to sequencing with Dye Terminator Cycle Sequencing FS kit (Perkin Elmer Co., Ltd.), thereby it was confirmed that the plasmid had the sequence encoding ~~the secreted~~secretory-~~form~~type OBM (~~nucleotide sequence:~~nucleotides 338-1355 ~~in~~of ~~Sequence~~SEQ ID ~~No.~~NO: 2, amino ~~acid sequence:~~acids 72--316 ~~in the Sequence~~of SEQ ID ~~No.~~NO: 1) ~~by sequencing using a dyeterminator cycle sequencing FS kit (Perkin Elmer Co.)~~. ~~This~~After the plasmid was digested with restriction enzymes, NheI and XhoI ~~to isolate~~, a fragment (1.0 kb) ~~containing the~~ corresponding to secrete~~d~~ory-~~form~~type OBM cDNA was collected by agarose gel electrophoresis. This fragment was inserted into ~~the~~a NheI/XhoI ~~fragment (10.4 kb) of an~~digested expression vector, pCEP4 (10.4 kb) (Invitrogen Co., Ltd.)~~, using a~~, by using the ligation kit, and ~~Escherichia~~*E. coli* DH5-~~α was~~α were transformed ~~using~~with the ~~reaction~~ligation product ~~thereof.~~. Plasmids ~~were~~was purified ~~by alkali-SDS method~~, from the ~~resulting~~ ampicillin -resistant strains obtained, by alkaline-SDS method, and digested with the restriction enzymes. Then the plasmid was analyzed so as to select ~~an Escherichia~~*E. coli* ~~strain having the secreted-form~~strains which had a plasmid for expressing secretory-type OBM ~~expression plasmid~~ (pCEP sOBM) with the ~~correct~~desired structure. ~~The~~An ~~Escherichia~~*E. coli* strain ~~containing~~having the pCEP sOBM was cultured, and the pCEP sOBM was purified ~~using~~therefrom with QIA~~-filter plasmid midi kit~~® Filter Plasmid Midi Kit (QIAGEN ~~Co~~CO., LTD.).

(2) Expression of ~~s~~Secrete~~d~~ory-~~form~~Type OBM

293-EBNA cells were suspended in IMDM containing 10% FCS (IMDM-10% FCS), and seeded ~~into~~in a collagen-coated 24- well plate~~-coated with collagen (manufactured by Sumitomo Bakelite Co~~SUMITOMO BAKELITE CO., ~~Ltd~~LTD.) ~~in a~~so that the cell ~~density~~concentration ~~of~~was 2~~×~~ X 10^5 cells/2 ml/well, and cultured overnight. The cells were transfected with 1 ~~μ~~μg of pCEP sOBM or pCEP4 using 4 ~~μ~~μl of ~~L~~lipofectamine ~~(Gibco Co.). After culturing for two days~~ (GIBCO CO., LTD.), and then cultured in 0.5 ml of ~~a~~serum-free IMDM or IMDM-10%~~-FCS~~ FCS for another 2 days. Thereafter, the conditioned medium was collected. Expression of ~~the secreted~~secretory-~~form~~type OBM in the conditioned medium was confirmed ~~as follows~~in the following manner. ~~Sodium hydrogen carbonate~~After sodium hydrogencarbonate was added to the conditioned medium ~~to a~~so that the final concentration ~~of~~was 0.1 M ~~and~~, the culture solution was added to a 96-well plate~~. The plate was allowed~~, and left to stand at 4°C overnight ~~at 4°C, thereby immobilizing~~. Then the OBM in the conditioned medium ~~on~~was immobilized in the 96-well plate. ~~The plate was filled with a Block Ace™~~This plate was left to stand for blocking at room temperature for 2 hours by use of BLOCKACE (Snow Brand Milk Products Co., Ltd.) solution diluted ~~four-fold~~ with PBS to be one forth concentration (B-PBS) ~~and allowed to stand for two hours at room temperature to block residual binding sites of the plate. After the addition to each well of 100~~ Then, 100 ~~μ~~μl of 3-100 ng/ml ~~of~~ OCIF ~~which was~~ diluted with B-PBS~~, the plate was allowed~~ was added to each well, and the wells were left to stand ~~for two hours~~ at 37°C~~, followed by~~ for 2 hours. After washing the plate with PBS containing 0.05% Tween 20 (PBS-T)~~. Then, 100~~, 100 ~~μ~~μl of ~~a~~ peroxidase-labeled ~~rabbit~~ anti-OCIF rabbit polyclonal antibody, which was described in WO 96/~~26217 which was~~26217, diluted with B-PBS was added to each well~~. After allowing~~, and the cells were left to stand at 37°C for ~~two~~2 hours ~~at 37°C, the wells were washed six times~~. After washing each well with PBS-T~~. Then~~ six times, ~~a~~100 μl of TMB solution (TMB Soluble Reagent, High Sensitivity, Scytek Co., Ltd.) was added ~~in the amount of 100 μl per well~~thereto and ~~allowed~~then left to stand at room temperature for about 10 minutes. Thereafter, ~~whereupon the reaction was terminated by the addition of~~ 100 ~~μ~~μl of ~~a termination solution~~ (Stopping Reagent, (Scytek Co., Ltd.) was added to each well. ~~Absorbance~~The absorbance of each well at

450 nm ~~of each well~~ was measured ~~by~~with a microplate reader. The results are shown in ~~Figure 12~~Fig. 12. In the plate in which ~~indicates that~~substances included in the ~~absorbance~~conditioned medium of the cells transduced by pCEP sOBM were immobilized, absorption at 450 nm increased ~~according to~~in the OCIF concentration ~~of the added OCIF in~~-dependent manner. On the other hand, in the ~~plate in which~~ plate in which substances included in the conditioned medium ~~of~~ of the cells ~~transfected with pCEP-sOBM was immobilized. On the other hand,~~ transduced by pCEP4 vector were immobilized, no increase in ~~absorbance~~absorption at 450 nm was ~~seen in the plate in which the conditioned medium of the cells transfected with vector pCEP4 was immobilized~~observed. ~~Figure~~Furthermore, Fig. 13 shows the results of ~~an experiment wherein~~experiments when the ~~proportion~~amount of the conditioned medium ~~which is used for~~applied to the immobilization was ~~changed~~varied within a range of 5- to 90% and a ~~specific~~constant concentration of OCIF (50 ng/ml) was futher added. ~~It can be seen that~~In the ~~absorbance~~plate in which substances included in the conditioned medium of the cells transduced by pCEP sOBM were immobilized, absorption at 450 nm increased ~~according~~corresponding to ~~the~~ an increase in the amount of the ~~proportion of~~conditioned medium. On the ~~conditioned medium in~~other hand, in the ~~plate wherein~~ plate in which substances included in the conditioned medium ~~of~~ of the cells ~~transfected with pCEPsOBM was immobilized, whereas~~transduced by pCEP4 vector were immobilized, no ~~such~~ increase in ~~absorbance was seen in the plate wherein the conditioned medium of the cells transfected with vector pCEP4 was immobilized~~absorption was observed. From these results, ~~it~~secretory-type OBM was confirmed ~~that secreted form OBM is~~to be produced ~~into~~in the conditioned medium of the cells transfeducted ~~with~~ by pCBEP sOBM.

~~<~~[Example 15~~>~~]

Expression of ~~t~~Thioredoxin-OBM ~~f~~Fusion ~~p~~Protein (Trx-OBM)

(1) Construction of a ~~thioredoxin~~Vector for Expressing Thioredoxin-OBM ~~f~~Fusion ~~p~~Protein (Trx-OBM) ~~expression vector~~

~~Ten~~10 ~~μ~~μl of ~~10~~10X ExTaq buffer (~~Takara Shuzo Co~~TAKARA SHUZO CO., LTD.), 8 ~~μ~~μl of 10 mM ~~dNTP (Takara Shuzo Co~~dNTPS (TAKARA SHUZO CO., LTD.), 77.5 ~~μ~~μl of sterilized distilled water, 2 ~~μ~~μl of ~~an aqueous~~pOBM291 solution ~~of~~

~~pOBM291~~ (10 ng/~~μ~~μl), 1 ~~μ~~μl of primer OBM3 (100 pmol/~~μ~~μl, ~~Sequence Table,~~ ~~Sequence~~SEQ ID ~~No.~~NO: 9), 1 ~~μ~~μl of primer OBMSalR2 (100 pmol/~~μ~~μl, ~~Sequence Table, Sequence~~SEQ ID ~~No.~~NO: 10)~~,~~ and 0.5 ~~μ~~μl of ExTaq (5~~-μ~~μ/~~μ~~μl) (Takara Shuzo Co., Ltd.) were mixed together, and ~~reacted~~then ~~(~~PCR ~~reaction)~~was conducted in a microtube ~~centrifuge tube~~ for centrifugation. After ~~reacting~~the reaction was carried out at 95°C for 5 minutes, ~~at~~ 50°C for ~~one~~1 second, ~~at~~ 55°C for ~~one~~1 minute, ~~at~~ 74°C for ~~one~~1 second~~,~~ and ~~at~~ 72°C for 5 minutes, ~~a~~the cycle reaction consisting of ~~a reaction~~ at 96°C for ~~one~~1 minute, ~~at~~ 50°C for ~~one~~1 second, ~~at~~ 55°C for ~~one~~1 minute, ~~at~~ 74°C for ~~one~~1 second~~,~~ and ~~at~~ 72°C for 3 minutes~~,~~ was repeated 25 times. ~~From the total reaction liquid~~ After gel electrophoresis through 1% agarose, an approximately 750 bp DNA fragment ~~of about 750 bp~~ was purified ~~by 1% agarose gel electrophoresis using~~from the whole reaction solution with QIAEX® II ~~g~~Gel ~~e~~Extraction ~~k~~Kit (QIAGEN Co., Ltd.). ~~The whole amount~~All of the purified DNA fragment was ~~digested~~cleaved with restriction enzymes Sa~~l~~lI and EcoRI (Takara Shuzo Co., Ltd.), and ~~subjected to an 1.5% agarose gel electrophoresis to purify a~~a DNA fragment ~~of~~ about 160 -bp ~~(Fragment 1), which was~~(fragment 1) was purified by gel electrophoresis through 1.5% agarose and dissolved in 20 ~~μ~~μl of sterilized distilled water. ~~In the same manner~~Similarly, ~~a DNA fragment of about 580 bp (Fragment 2) obtained by digesting~~ 4 ~~μ~~μg of pOBM291 and 2 μg of pTrxFus (Invitrogen Co., Ltd.) were cleaved with restriction enzymes ~~BamH1 and~~ BamHI/EcoRI and BamHI/SalI (Takara Shuzo Co., Ltd.) ~~and a~~, respectively. A DNA fragment ~~of~~ about 580-bp (fragment 2) and an approximately 3.6 -kb DNA fragment (~~F~~fragment 3) ~~obtained by digesting 2 μg of pTrXFus (Invitrogen Co.) with restriction enzymes BamHI and SalI (Takara Shuzo Co.)~~ were ~~respectively~~ purified therefrom, respectively, and dissolved in 20 ~~μ~~μl of sterilized distilled water. ~~The QIAEXII gel extraction kit~~QIAEX® II Gel Extraction Kit was used for for purifying the ~~purification of DNA~~ fragments. Fragments ~~1~~ 1, 2 and 3 were ligated by incubating them using DNA Ligation Kit Ver. 2 (Takara Shuzo Co., Ltd.) at 16°C for 2.5 hours ~~using DNA ligation kit ver. 2 (Takara Shuzo Co.)~~. ~~Using the ligation reaction liquid~~Then, ~~Escherichia~~*E. coli* ~~strain~~ GI724 cells (Invitrogen Co., Ltd.) ~~was~~were transformed ~~according to~~with the ligation product in accordance with the method described in ~~the Instruction Manual of~~an instruction manual attached to ThioFusion Expression System (Invitrogen Co., Ltd.). ~~A~~

~~microorganism strain with~~Among the resulting ampicillin-resistant transformants, one having a plasmid, in which ~~the~~an OBM cDNA fragment (~~nucleotide sequence: 350--1111 in the Sequence~~of SEQ ID ~~No.~~NO: 2, ~~amino acid sequence~~corresponding to: 76-316 ~~in the Sequence~~of SEQ ID ~~No.~~NO: 1) ~~is fused in frame~~was linked to a thioredoxin gene in the same reading frame and was selected ~~from the resulting ampicillin resistant transformants by the~~after analysis of ~~restriction maps~~DNA fragment map obtained by ~~digestion with~~ restriction ~~enzymes~~enzyme cleavage and DNA ~~sequence determination~~sequencing. The ~~microorganism~~obtained strain ~~thus obtained~~ was ~~named~~referenced as GI724/pTrxOBM25.

(2) Expression of OBM in ~~Escherichia~~*E. coli*

The GI724/pTrxOBM25 strain and the GI724 ~~containing~~strain having pTrxFus (GI724/pTrxFus) were ~~respectively~~ cultured ~~six hours with shaking at 30°C~~ in 2 ml of RMG-Amp ~~culture~~ medium (0.6% Na_2HPO_4, 0.3% KH_2PO_4, 0.05% NaCl, 0.1% NH_4Cl, 1.2% casamino acid (Difco Co., Ltd.), 1% glycerol, 1 mM $MgCl_2$, ~~and~~ 100 ~~μ~~μg/ml ampicillin (Sigma Co., Ltd.), pH 7.4) with shaking at 30°C for 6 hours. ~~The broth~~ 0.5 ml of the ~~broth~~cell suspension was added to 50 ml of Induction ~~culture~~ medium (0.6% Na_2HPO_4, 0.3% KH_2PO_4, 0.05% NaCl, 0.1% NH_4Cl, 0.2% casamino acid, 0.5% glucose, 1 mM $MgCl_2$, 100 ~~μ~~μg/ml ampicillin, pH 7.4) and cultured with shaking at 30°C. ~~When OD_{600nm} reached about 0.5,~~ L-tryptophan was added ~~to a~~so that the final concentration ~~of~~was 0.1 mg/ml~~, followed by shaking~~ when the ~~culture~~value at OD_{600} became about 0.5, and the cells were further cultured at 30°C for ~~an additional~~ 6 hours. The ~~culture~~cell ~~broth~~suspension was centrifuged at ~~3000~~3,000 ~~×~~X g ~~to collect~~and the collected cells~~,~~ ~~which~~ were then suspended in 12.5 ml of PBS (10 mM ~~phosphate~~phosphoric acid buffer, 0.15 M NaCl, pH 7.4). The suspension was subjected to ~~an ultrasonic generator~~ultrasonication using a ultrasonicator (Ultrasonics Co., Ltd.) ~~to~~so ~~disrupt~~that the ~~cells. The disrupted~~ cells were crushed and then centrifuged at ~~7000 ×~~7,000 X g for 30 minutes ~~to obtain a~~. The recovered supernatant ~~liquid~~was used as ~~a~~ soluble protein fraction. ~~Ten~~10 ~~μ~~μl of ~~this~~e soluble protein fraction solution was subjected to SDS polyacrylamide (10%) electrophoresis under reducing conditions. As a result, a band ~~with~~having a molecular weight of about 40 kDa ~~which~~ was observed in the soluble protein fraction solution of GI724/pTrxOBM25, while not ~~detected~~observed in ~~the~~

soluble protein fraction solution of GI724/pTrxFus ~~was found in the soluble protein fraction of GI724/pTrxOBM25 (Figure~~Fig. 14). ~~Accordingly~~Thus, it was confirmed that ~~a~~the thioredoxin-OBM fusion protein (Trx-OBM) ~~of thioredoxin and OBM~~ was expressed in ~~Escherichia~~*E. coli*.

(3) Binding ~~capability~~Ability of Trx-OBM to OCIF

~~Binding of~~In the following experiment, it was confirmed that the expressed Trx-OBM bound to OCIF ~~was confirmed according to the following experiment~~. Anti-thioredoxin antibody (Invitrogen Co., Ltd.) ~~which~~ was diluted ~~to 5000-fold~~ with 10 mM sodium ~~hydrogen carbonate~~hydrogencarbonate solution so that the concentration was 1/5,000. 100 μl thereof was added to each well of a 96-well immunoplate (Nunc Co., Ltd.) ~~in the amount of 100 μl per well. After being allowed~~and then left to stand at 4°C overnight ~~at 4°C, the liquid in the wells~~. After the solution in each cell was discarded. ~~Two hundred~~, 200 ~~μμ~~l ~~of a solution prepared by diluting Block Ace™~~ of 1/2 concentration of BLOCKACE (Snow Brand Milk Products Co., Ltd.) ~~two-fold~~diluted with PBS (BA-PBS) was added to each well. ~~After being allowed~~ and then left to stand ~~for one hour~~ at room temperature~~,~~ for 1 hour. After the solution was discarded ~~and~~, 100 μl of the soluble protein ~~fractions originating~~fraction solution derived from ~~the above-described~~ GI724/pTrxOBM25 ~~or~~which was diluted stepwise with BA-PBS and 100 μl of that derived from GI724/pTrxFus~~, each~~ which was diluted stepwise with BA-~~PBS in various concentrations~~BPB were added to ~~each well in the amount of 100 μl. After being allowed~~wells and left to stand ~~for two hours~~ at room temperature~~,~~ for 2 hours, respectively. After washing each ~~well was washed~~well three times with PBS-T ~~and charged with~~, 100 ~~μμ~~l of OCIF (100 ng/ml) ~~which was~~ diluted with BA-PBS. ~~After being allowed~~ was added to each well and left to stand ~~for two hours~~ at room temperature~~, each~~ for 2 hours. After washing each well ~~was washed~~ three times with PBS-T ~~and charged with~~, 100 ~~μμ~~l of peroxidase- labeled ~~rabbit~~ anti- OCIF rabbit polyclonal antibody (~~described~~desribed in WO 96/~~26217~~)26217, which was diluted ~~2,000-fold~~ with BA-PBS. ~~After being allowed~~ so that the concentration was 1/2,000, was added to each well and left to stand ~~for two hours~~ at room temperature~~, each~~ for 2 hours. After washing each well ~~was washed~~ six times with PBS-T ~~and charged with~~, 100 ~~μμ~~l of TMB solution (TMB Soluble Reagent, High Sensitivity, Scytek Co.~~). After being~~

~~allowed~~, Ltd.) was added thereto and then left to stand at room temperature for about 10 minutes~~ at room temperature~~. Thereafter,~~ each well was charged with~~ 100 ~~μ~~μl of ~~termination solution (~~Stopping Reagent~~,~~ (Scytek Co., Ltd.) was added thereto. Absorbance of each well at 450 nm was measured ~~by~~with a microplate reader. The results are shown in ~~Figure~~Fig. 15. ~~There was~~When the concentration of the soluble protein fraction solution derived from GI724/pTrxFus increased, the absorbance increased in a concentration (of the added solution)-dependent manner, while no difference in absorbance was observed between ~~the sample with~~when the soluble protein fraction ~~originating~~solution derived from GI724/pTrxFus was added ~~thereto~~ and ~~the sample without the addition of this~~when said soluble protein fraction solution was not added. ~~On the other hand~~Furthermore, ~~the absorbance increased in the samples to which the soluble protein fraction originating from GI724/pTrxOBM25 was added in proportion to the concentration of the soluble protein fraction~~Fig. ~~The~~ 16 shows the results of ~~the other experiment wherein~~experiments when the dilution rate of the soluble ~~protein~~ fraction solution was ~~maintained~~kept constant (1%) ~~while adding~~and OCIF diluted stepwise with BA-PBS ~~in different concentrations~~ (0-100 ng/ml) ~~are shown in Figure 16.~~was further added. ~~It can be seen that the absorbance remained~~Absorbance was kept low ~~at any concentrations~~regardless of the concentration of OCIF ~~in samples using a~~when soluble protein fraction ~~originating~~solution derived from GI724/pTrxFus was added. However,~~ whereas the~~ absorbance was increased in ~~proportion to the~~an OCIF concentration ~~in the samples to which the~~-detendent manner when soluble protein fraction ~~originating~~solution derived from GI724/pTrxOBM25 was added. ~~Based on these results~~Thus, it was confirmed that the Trx-OBM ~~which is~~ produced ~~from~~in GI724/pTrxOBM25 ~~has a capability of binding~~had an ability to bind OCIF.

(4) Large- ~~s~~Scale ~~cultivation~~ Culture of ~~Escherichia~~*E. coli* ~~which produces~~Producing Trx-OBM

GI724/pTrxOBM25 ~~cells were~~was spread on an RMG-Amp agar medium (0.6% Na_2~~PO~~HPO_4, 0.3% KH_2PO_4, 0.05% NaCl, 0.1% NH_4Cl, 2% casamino acid, 1% glycerol, 1 mM $MgCl_2$, 100 ~~μ~~μg/ml ampicillin, 1.5% agar, pH 7.4) ~~using~~with a platinum ~~transfer~~ loop. ~~The cells were~~ and cultured at 30°C overnight ~~at 30°C~~. The ~~cultured~~ cells were suspended in 10 ml of Induction medium. ~~The~~5 ml of the suspension was added ~~5 ml for~~

~~each~~ to two of 2~~-l Erlenmeyer~~L conical flasks containing 500 ml of Induction medium and cultured ~~at 30°C with~~by shaking at 30°C. ~~When the OD_{600nm} reached about 0.5,~~ L-tryptophan was added ~~to a~~so that the final concentration ~~of~~was 0.1 mg/ml~~. Culturing with~~ when OD_{600} value became about 0.5, and then the cells were further cultured by shaking ~~was~~at ~~continued~~30°C for ~~six~~6 hours~~ at 30°C~~. The ~~culture~~cell ~~broth~~suspension was centrifuged at 3,000 X g for 20 minutes~~ at 3000 x g to collect~~, and the cells~~, which~~ were collected and then suspended in 160 ml of PBS. The suspension was subjected to ustrasoniaction using an ~~ultrasonic generator~~ultrasonicator (Ultrasonics Co., Ltd.) ~~to disrupt the~~for crushing cells~~. The supernatant liquid was~~, and then centrifuged at 7,000 X g for 30 minutes~~ at 7000 x g to obtain a~~. Thereafter, the supernatant was recovered as soluble protein fraction.

(5) Preparation of OCIF-immobilized ~~a~~Affinity ~~c~~Column

~~Two~~2 g of TSKgel AF-T~~ol~~resyl ~~Toyopal~~TOYOPAL 650 (~~Tosoh Corp~~Toso Co., Ltd.) and 40 ml of 1.0 M potassium phosphate buffer (pH 7.5) containing 35.0 mg of recombinant OCIF~~, which was~~ prepared ~~according to the~~by a method described in WO 96/~~26217,~~26217 were mixed~~. The mixture was~~ together and gently shaken at 4°C overnight ~~at 4°C to effect~~so as to cause a coupling reaction.~~ The reaction mixture was centrifuged to remove~~ After the supernatant~~. To inactivate excess active residues~~ was removed by centrifugation, 40 ml of 0.1 M Tris-~~HCl~~hydrochloric acid buffer (pH 7.5) was added to the precipitated carrier, and the mixture was gently shaken at room temperature for ~~one~~1 hour~~. The carrier, in a column was washed~~ order to inactivate an excess amount of active groups thereon. After washing the column with both 0.1 M glycine-~~HCl~~hydrochloric acid buffer (pH 3.3) containing 0.01% Polysorbate 80~~ and~~/0.2 M NaCl and 0.1 M sodium citrate buffer (pH 2.0) containing 0.01% Polysorbate 80~~ and~~/0.2 M NaCl~~. The carrier in the column was equilibrated by charging,~~ the column was washed twice with 10 mM sodium phosphate buffer (pH 7.4) containing 0.01% Polysorbate ~~80.~~80 and equilibrated therewith.

(6) Purification of Trx-OBM ~~u~~Using OCIF-~~i~~Immobilized ~~a~~Affinity ~~c~~Column

~~Unless otherwise indicated, purification~~Purification of Trx-OBM was carried out at 4°C unless otherwise stated. The above~~ mentioned~~ OCIF-immobilized affinity carrier (10 ml) and the above soluble protein fraction solution (120 ml) ~~prepared~~described in

Example 15-(4) were mixed together. The mixture was gently shaken ~~overnight at 4°C~~ in four 50- ml centrifugeation tubes ~~using~~with a rotor at 4°C overnight. ~~An~~The carrier in the mixture was embeded in Econo-~~column~~™Column (Bio-Rad Co., Ltd., internal diameter: 1.5 cm, length: 15 cm~~, manufactured by BioRad Co.) was filled with the carrier in the mixture~~. The column was ~~charged~~washed with 300 ml of PBS containing 0.01% Polysorbate 80, 100 ml of 10 mM sodium phosphate buffer (pH 7.0) containing 0.01% Polysorbate 80 and 2 M NaCl~~,~~ and 100 ml of 0.1 M glycine-~~HCl~~hydrochloric acid buffer (pH 3.3) containing 0.01% Polysorbate 80 and 0.2 M NaCl~~, in that order.~~. ~~Next, proteins adsorbed in~~Then, protein was eluted from the column ~~were eluted~~ with 0.1 M sodium citrate buffer (pH 2.0) containing 0.01% Polysorbate 80 and 0.2 M NaCl. ~~The~~5 ml eluate ~~was~~ fractions were collected ~~in 5 ml portions.~~. ~~Each fraction thus collected was immediately neutralized with addition~~Immediately, a 10% volume of 2 M Tris ~~buffer~~solution (pH 8.0) was added for neutralization. ~~Presence~~The presence or absence of Trx-OBM in each fraction of the ~~eluted fractions~~eluate was ~~determined according to~~examined in accordance with the above method ~~previously~~as described in Example 15-(3) (the ~~binding capability~~ability to bind OCIF). ~~The fractions~~Fractions containing Trx-OBM were collected and further purified ~~further~~.

(7) Purification of Trx-OBM by ~~g~~Gel ~~f~~Filtration

~~About~~Using Centriplus® 10 and Centricon® 10 (Amicon Co., Ltd.), about 25 ml of the above Trx-OBM ~~fractions obtained in~~fraction of Example 15 -(6) was concentrated by centrifugation to a final volume of about 0.5 ml ~~by centrifuge using Centriplus 10 and Centricon 10 (Amicon Co.)~~. This sample was ~~applied~~subjected to ~~a~~ Superose® 12 HR 10/30 column (1.0 ~~x~~X 30 cm, Pharmacia Co., Ltd.) previously equilibrated with PBS containing 0.01% Polysorbate 80. ~~For the separation,~~The column was developed using PBS containing 0.01% Polysorbate 80 ~~was used~~ as a mobile phase at a flow rate of 0.25 ml/min~~. The~~ and 0.25 ml eluate fractions were collected from the column ~~was collected in 0.25 ml portions.~~. ~~The~~ Trx-OBM in the ~~thus collected~~ fractions was detected by the ~~same~~ method ~~as previously~~ described in Example 15-(3) and by SDS-polyacrylamide electrophoresis (gradient gel of 10- to 15% polyacrylamide ~~gel~~, Pharmacia Co., Ltd.) using Phast System (Pharmacia Co., Ltd.) and silver staining. Fractions (Fr. 20- to 23) containing purified Trx-OBM were collected and ~~the~~ subjected to measurement of Trx-

OBM protein concentration ~~of Trx-OBM was determined~~. The measurement ~~of the protein concentration~~ was carried out with DC-protein assay kit (Bio-Rad Co., Ltd.) using bovine serum albumin as a standard ~~substance using DC-Protein assay kit (BioRad Co.).~~ <[Example 16>]

~~Osteoclast formation-inducing activity of OBM~~

Osteoclastogenesis Promoting Activity of OBM

COS-7 cells were transfected with pOBM291 and pcDL-SR ~~α~~α296 ~~were respectively transfected into COS-7 cells~~ using ~~L~~lipofectamine (Gibco BRL Co., Ltd.), respectively. ~~The~~After the cells were cultured in DMEM containing 10% FCS for ~~one~~1 day, ~~trypsinized~~they were treated with trypsin, ~~plated on~~and seeded in a 24-well plate, in which a cover ~~slips~~glass (15 mm round ~~shape, manufactured by~~ Matsunami Co., Ltd.) ~~in 24-well plates at~~was seated, so that the concentration became 5 X 10^4 cells ~~per~~/well~~, and~~. The cells were then cultured for ~~2~~another two days. ~~The~~After washing the culture plate ~~was washed~~ once with PBS~~. The cells were fixed with,~~ PBS containing 1% paraformaldehyde was added thereto, and the cells were incubated at room temperature for 8 minutes and fixed. ~~The~~After washing the plate ~~on~~in which the ~~fixed~~ cells were ~~attached was washed 6~~fixed six times with PBS, ~~then~~ 700 ~~μ~~μl of 1 X 10^6 cells/ml suspension of mouse spleen ~~cells suspended at 1x10^6/ml~~cell in ~~α~~α-MEM containing 10^{-8} M of the active-form of vitamin D_3, 10^{-7} M dexamethasone~~,~~ and 10% bovine fetal ~~bovine~~ serum ~~were~~was added to each ~~well~~cell. A Millicell® PCF (Millipore Co., Ltd.) was set ~~in~~on each well, and a700 μl of 4 X 10^4 cells/ml suspension of ST2 cells in the above-~~mentioned culture~~ medium ~~(4 x 10^4/ml) were~~was added~~, 700 μl per well, into~~ to the Millicell® PCF ~~followed by incubation~~and cultured at 37°C for 6 days. After the culture, the Millicell® PCF was removed, and the plate was washed once with PBS~~, and the~~. The cells were fixed with acetone-ethanol solution (50:50) for ~~one~~1 minute~~. Then~~, and then only the cells ~~exhibiting~~showing tartaric acid- resistant acid phosphatase activity (TRAP activity), ~~which is~~ a specific marker for osteoclasts, were ~~selectively~~ stained ~~using LEUKOCYTE ACID PHOSPHATASE~~with leukocyte acid phosphatase kit (Sigma Co., Ltd.). As a result of ~~microscopic~~ observation~~, TRAP-positive cells were not detected in the wells in which COS-7 cells transfected with pcDL-SR α296 were fixed. In contrast~~ using a microscope, 45~~±~~ ± 18 (average ~~±~~± standard deviation, n = 3) TRAP positive cells

were observed in the wells in which ~~COS-7 cells~~ pOBM291-transfected COS-7 cells were fixed, while no cells showing TRAP activity were detected in the wells in which pcDL-SR α296-transfected ~~with pOBM291~~COS-7 cells were fixed. ~~Moreover~~Furthermore, it was also confirmed that calcitonin bound ~~to these~~said TRAP positive cells. ~~Based on these findings~~Thereby, it ~~has been proven~~was revealed that OBM ~~has~~had an activity to promote osteoclast formation ~~inducing activity~~.

<[Example 17>]

~~Osteoclast formation inducing activity~~**Osteoclastogenesis Promoting Activities of Trx-OBM and ~~s~~Secrete~~dory form~~type OBM**

Mouse spleen cells were suspended in ~~e~~α-MEM containing 10^{-8} M of the active-form of vitamin D_3, 10^{-7} M dexamethasone~~,~~ and 10% bovine fetal ~~bovine~~ serum ~~at~~in a concentration of 2 ~~x~~X 10^6 cells/ml~~. The~~, and 350 μl of this suspension was added to each well of a 24 -well plate ~~in the amount of 350 μl per well~~. ~~Each well was then charged with~~ 350 ~~μ~~μl of ~~a~~the solution ~~prepared~~ (40 ng/ml) obtained by diluting the purified Trx-OBM with the above ~~mentioned culture~~ medium ~~(40 ng/ml)~~, 350 ~~μ~~μl of solution ~~prepared~~obtained by ~~10 fold~~ diluting the conditioned medium ~~which was produced by culturing~~of 293-EBNA cells~~, in~~ (which were transduced by pCEP sOBM or pCEP4 ~~were transfected,~~cultured in IMDM-10% FCS~~,~~ with the above ~~mentioned culture~~ medium~~,~~) so that the concentraion was 1/10, or 350 ~~μ~~μl ~~only~~ of the above ~~mentioned culture~~ medium~~.~~ ~~The~~ alone was added to each well. Then, a Millicell® PCF (M~~e~~illipore Co., Ltd.) was set on each well, ~~to which~~and 600 ~~μ~~μl of ~~ST2 cells which was suspended~~4 X 10^4 cells/ml suspension of ST2 cell in the above ~~mentioned culture~~ medium ~~(4 x 10^4/ml)~~ were added to the Millicell® PCF. After the cells were cultured for ~~six~~6 days, the Millicell® PCF was removed~~. The~~, and the plate was washed once with PBS ~~and~~. When the cells were fixed with acetone-ethanol solution (50: 50) for ~~one~~1 minute~~. Then~~, only the cells ~~exhibiting the~~showing tartaric acid resistant acid~~ic~~ phosphatase activity (TRAP activity) were ~~selectively~~ stained ~~using~~with LEUKOCYTE ACID PHOSPHATASE kit (Sigma Co., Ltd.). ~~The result of microscopic~~Through observation ~~revealed that no cells exhibiting the TRAP activity were detected in the wells to which no Trx-OBM was added~~under a microscope, ~~whereas~~ 106~~+~~ ± 21 (average ~~+~~± standard deviation, n = 3) TRAP- positive cells were observed in the wells ~~to which~~when Trx-OBM was added,

while no cells showing TRAP activity were detected in the wells when not added. Similarly, ~~while no cells exhibiting TRAP activity were detected in the wells to which conditioned medium of 293-EBNA transfected with pCEP4 had been added,~~ 120~~±~~ ± 31 (average ~~±~~± standard deviation, n = 3) TRAP positive cells were observed in the wells ~~to~~when ~~which~~the conditioned medium of 293-EBNA ~~transfected with pCEPsOBM had been~~cells transduced by pCEP-sOBM was added, while no cells showing TRAP activity were detected in the wells when not added. ~~Moreover~~Furthermore, it was ~~also~~ confirmed that calcitonin b~~i~~ounds to these TRAP positive cells. ~~These results have proven~~Thereby, it was revealed that Trx-OBM and secrete~~d~~ory ~~form~~type OBM ~~exhibit~~had an activity to promote osteoclast formation ~~inducing activity~~.

~~<~~[Example 18~~>~~]

Identity of the ~~p~~Protein OBM ~~e~~Expressed by the cDNA of the ~~p~~Present ~~i~~Invention and ~~the natural type~~Natural-Type OCIF- ~~b~~Binding ~~p~~Protein of the ~~p~~Present ~~i~~Invention

~~(1)~~

(1) ~~p~~Preparation ~~of rabbit anti-OBM~~ of Anti-OBM Rabbit ~~p~~Polyclonal ~~a~~Antibody

Three male Japanese white rabbits (weight: 2.5- to 3.0 kg, ~~supplied~~purchased ~~by~~from Kitayama Labes~~t~~h Co., Ltd.) were ~~immunized~~subjected to hypodermic immunization with ~~the purified OBM (thioredoxin-OBM fusion protein) produced according to the method in Examples 14(6) and 14(7) by subcutaneously injecting~~ 1 ml~~/dose~~ of emulsion prepared by mixing 200 ~~μ~~μg/ml of ~~the~~ purified OBM (thioredoxin-OBM fusion protein), which was obtained in accordance with ~~equal volume~~the methods described in Examples 14-(6) and 14-(7), with 200 μg/ml of Freund's complete adjuvant (~~DIFCO~~Difco Co.~~)~~, ~~six~~Ltd.). The immunization was carried out 6 times~~, once a~~ in total with one-week~~. Ten days after~~ interval each, and all the blood was collected from the rabbits on the 10th day counted from the last immunization~~, the rabbits were exsanguinated~~. ~~Antibody~~An antibody was purified from the fractionated serum ~~as follows~~in the following manner. ~~Ammonium sulfate was added to the~~That is, the antiserum ~~which was~~ diluted ~~two-fold~~ with PBS to ~~a~~be 1/2 concentraion, and ammonium sulfate was added thereto so that the final concentration ~~of~~was 40% (w/v~~%~~). ~~After being allowed~~ Then, the antiserum was left to stand at 4°C for ~~one~~1 hour ~~at 4°C, the mixture~~

~~was~~and centrifuged at 8,000 X g for 20 minutes~~ at 8000 x g to obtain a precipitate~~. ~~The~~Thereafter, the precipitate was collected and dissolved in a small ~~am~~liqu~~no~~t of PBS, and then dialyzed against PBS at 4°C~~, and loaded to~~. The resulting solution was charged onto a Protein G-Sepharose® column (~~manufactured by~~ Pharmacia Co., Ltd.). After ~~washed~~washing the column with PBS,~~ the adsorbed~~ immunoglobulin G adsorbed was eluted with 0.1 M glycine-~~HCl~~hydrochloric acid buffer ~~solution~~ (pH 3.0)~~. The eluate~~, and the pH thereof was immediately ~~neutralized~~adjusted to be neutral with 1.5 M Tris-~~HCl~~hydrochloric acid buffer (pH 8.7). After ~~dialyzing~~ the eluted protein ~~fractions~~fraction was dialyzed against PBS,~~ the~~ absorbance at 280 nm was measured ~~to determine the protein~~and its concentration was determined ($E^{1\%}$ 13.5). ~~Anti~~Horseradish peroxidase-labeled anti-OBM antibody ~~labeled with horseradish peroxidase~~ was prepared ~~using a maleimide-activated paroxidase kit~~with Maleimide Activated Peroxidase Kit (Pierce Co., Ltd.)~~ as follows~~. That is, 80 ~~μ~~μg of N-succinimide-S-~~acetyl thioacetic~~acetylthioacetic acid was added to 1 mg of the purified antibody and ~~reacted~~allowed to react at room temperature for 30 minutes. ~~Five~~5 mg of hydroxylamine was added ~~to~~thereto for deacetylation, and then the~~ resulting mixture to deacetylate the antibody. The~~ modified antibody was fractionated by using a polyacrylamide desalting column. The protein fractions ~~were~~was mixed with 1 mg of maleimide- activated peroxidase and ~~reacted for one hour~~allowed to react at room temperature ~~to obtain~~for 1 hour, and then the enzyme-labeled antibody was obtained.

~~(2) Capability of rabbit anti-OBM polyclonal antibody to inhibit specific binding~~ Inhibition of Specific Binding of the ~~protein (OBM) expressed~~Protein Expressed by the cDNA of the ~~present invention~~Present Invention (OBM) or ~~the natural type protein~~Natural-Type Protein of the ~~p~~Present ~~i~~Invention ~~with~~ to OCIF by Anti-OBM Rabbit Polyclonal Antibody

~~Purified~~2 μg/ml of purified OBM (thioredoxin-OBM fuse~~d~~ion protein) obtained ~~according to~~in accordance with the methods described in ~~the~~ Examples 15-(6) and 15-(7) and ~~the~~2 μg/ml of natural -type purified OCIF- binding protein of ~~the~~ Example 2-(4) were dissolved ~~respectively~~ in 0.1 M sodium ~~carbonate buffer to a concentration of 2 μg/ml~~hydrogencarbonate, respectively. ~~An aliquot~~ 100 μl of each solution was added ~~100 μl per~~to each well ~~respectively to~~of a 96-well immunoplate (~~manufactured by~~ Nunc

Co.), Ltd. The plate was allowed) and then left to stand at 4°C overnight at 4°C. 200 μl of 50% Block AceBLOCKACE was added to each well and the plate was allowedleft to stand at room temperature for one1 hour. After washing each wellwells three times with PBS containing 0.1% Polysolrbate 20 (P20-PBS), 100200 μlμg/ml of rabbit anti-OBM rabbit antibody solution which was dissolved in 25% Block Ace preparedBLOCKACE diluted with P20-PBS to a concentration, and 100 μl of 200 μg/mlthe antibody solution or 100 μl of 25% Block Ace (containing noBLOCKACE without antibody) was added to each well, followed by incubation and incubated at 37°C for one1 hour. Each well was washedAfter washing wells three times with P20-PBS and charged with, 100 μμl/well of amedium for the binding test solutionexperiment (P20-PBS containing 0.2% calfbovine serum albumin, 20 mM Hepes, and 0.1 mg/ml hHeparin) to whichcontaining 20 ng/ml of the ^{125}I-labeled OCIF described in the Example 8-(3) was added thereto. AlternativelyFurthermore, each well was charged with 100 μμl/well of anothermedium for the binding test solutionexperiment containing 8 μμg/ml of unlabeled OCIF in addition to 20 ng/ml of the ^{125}I-labeled OCIF was added to other wells. After incubating these immunoplates at 37°C for one1 hour, the each wells were was washed six times with P20-PBS six times. The amount of ^{125}I in each well was measured bywith a gamma counter. The results are shown in FigureFig. 17. As shown in the figure, both the purifiedFig. 17, neither OBM expressed usingobtained by expressing the cDNA of the present invention and the protein that specifically bindsubsequently purifying or the natural -type OCIF specifically binding protein of the present invention do not bind to, which specifically binds OCIF, bound the ^{125}I-labeled OCIF at all, when they were treated with the rabbit anti-OBM polyclonal antibody, whereas both proteins boundrabbit antibody. On the other hand, it was confirmed that both proteins bound to the ^{125}I-labeled OCIF when untreated not treated with the antibody. The binding of both proteins to ^{125}I-labeledsaid antibody. Furthermore, it was also revealed that bindings of both proteins to OCIF was confirmed to be clearly were specific, because those binding since the bindings arewere significantly inhibited by the addition of a 400-fold higer concentration of unlabelled OCIF (8 μμg/ml). Based onFrom the above results described above, it was revealed that the rabbit anti-OBM rabbit polyclonal antibody recognizesd both the OBM which iswas thea protein expressed usingby the cDNA of the present invention and the

natural-type OCIF- binding protein of the present invention, and ~~it inhibits the~~inhibited specific binding of ~~these~~both proteins ~~with~~to OCIF.

~~<~~[Example 19~~>~~]

Cloning of ~~h~~Human OBM cDNA

(1) Preparation of ~~m~~Mouse OBM ~~p~~Primer

~~The~~For screening of human OBM cDNA, a mouse OBM ~~primers prepared according to~~primer prepared in accordance with the method of the ~~Examples (~~above Example, OBM #3 and OBM~~#8) described above, were used for screening of human OBM cDNA~~ #8 were used. ~~The~~Sequences ~~sequences~~thereof are shown in ~~the Sequence Table, Sequence~~SEQ ID ~~No.~~NO: 9 and ~~No. 6, respectively.~~SEQ ID NO: 6.

(2) Acquisition of Human OBM cDNA Fragments by PCR

~~(2) Isolation of~~A human OBM cDNA fragment ~~by PCR Human OBM cDNA fragments were~~was obtained by PCR method using ~~the mouse OBM cDNA primers prepared in (1) above and~~ Human Lymph Node Marathon ready cDNA (Clontech Co., Ltd.) which was a human lymph node derived cDNA library as a ~~template~~mold and using the mouse OBM cDNA primer prepared in the above (1).

The following are the conditions used for PCR ~~were shown as follows:~~.

10 ~~x~~X EX Taq buffer (Takara Shuzo Co.,	
~~2~~2.0 ~~μ~~μl	
2.5 mM dNTP	1.6 ~~μ~~μl
cDNA solution	~~1~~1.0 ~~μ~~μl
EX Taq (Takara Shuzo Co., Ltd.)	0.2 ~~μ~~μl
Distilled ~~w~~Water	14.8 ~~μ~~μl
40 ~~μ~~μM ~~p~~Primer OBM #3	
0.2 ~~μ~~μl	
40 ~~μ~~μM ~~p~~Primer OBM #8	
0.2 ~~μ~~μl	

~~These~~After the above solutions were mixed together in a microfuge tube ~~and pre-incubated~~, PCR was conducted under the following conditions. A pretreatment was

carried out at 95°C for 2 minutes, ~~followed by 40 cycles of a three-stage~~then the cycle reaction consiste~~d~~ing of ~~reactions at~~ 95°C for 30 seconds, ~~at~~ 57°C for 30 seconds~~,~~ and ~~at~~ 72°C for 2.5 minutes~~. After the reaction~~ was repeated 40 times, and the solution was incubated at 72°C for an approx. 5 minutes ~~at 72°C and a portion~~. A subfraction of the ~~solution was subjected to~~reaction product and run through agarose by electrophoresis ~~on an agarose gel. A~~ detected an approximate 690 bp DNA fragment ~~(about 690 bp)~~ amplified ~~by the~~with the above mouse OBM cDNA primers ~~described above was detected~~.

(3) Purification of ~~the human~~Human OBM cDNA ~~fragment amplified~~Amplified by PCR and ~~d~~Determination of ~~the nucleotide sequence~~Nucleotide Sequence

The human OBM cDNA fragments obtained in ~~the~~ Example 19-(2) ~~was~~were separated by agarose gel electrophoresis ~~on an agarose gel~~ and ~~further~~then purified ~~using~~by use of a QIAEX® gel extraction kit (~~Qiagen~~QIAGEN Co., Ltd.). ~~PCR was again performed using the~~ By use of the purified human OBM cDNA fragments as ~~a template and the~~templates, PCR was conducted again by use of the above mouse OBM cDNA ~~primers described above,~~primer so as to ~~produce~~prepare a large ~~quantity~~amount of ~~the~~ human OBM cDNA ~~fragment. The DNA fragment was~~fragments which were then purified by ~~a~~use of the QIAEX® gel extraction kit ~~in the same manner as above~~. The nucleotide sequence of the purified human OBM cDNA fragment was determined ~~using~~by use of a Taq Dye Deoxy Terminator Cycle Sequencing FS kit (Perkin Elmer Co., Ltd.) using OBM #3 ~~or~~and OBM #8 (~~Sequence~~SEQ ID ~~No.~~NO: 9 and ~~No. 6~~SEQ ID NO: 6, respectively) as ~~a primer~~primers. ~~When compared with the~~Comparing the nucleotide sequence of the human OBM cDNA fragment with the corresponding ~~area~~part of the mouse OBM cDNA, ~~the nucleotide sequence of the human OBM cDNA fragment showed 80.7%~~they share a homology ~~with that of the mouse OBM cDNA~~80.7%.

(4) Screening ~~of a full-length human~~for Full Length Human OBM cDNA by ~~hybridization using the human~~Hybridization With Human OBM cDNA ~~fragment (about~~Fragments With a Length of About 690 bp~~)~~ as ~~a probe~~Probes

~~A full-length~~ The human OBM cDNA ~~was screened using the human OBM cDNA fragment (~~fragments, with a length of about 690 bp~~) that was~~, purified in ~~the~~ Example 19-(3) ~~and~~were labeled with [~~α~~α-^{32}~~p~~P] dCTP ~~using~~by use of a

~~Megaprime~~MEGA PRIME DNA ~~L~~labeling kit (Amersham Co., Ltd.), and full length human OBM cDNA was screened. As an object to be screened, a Human Lymph Node 5' -STRETCH PLUS cDNA library (Clontech Co., ~~the U.S~~Ltd.~~A~~, USA) was ~~screened using the DNA probe~~used. ~~According to the manufacturer's~~In accordance with a protocol issued by the company, after *Escherichia coli* C600 Hfl was infected with ~~the~~ recombinant phage at 37°C for 15 minutes~~ at 37°C. The infected~~, the *Escherichia coli* was added to an LB agar medium (1% trypton, 0.5% yeast extract, 1% NaCl, 0.7% agar) ~~which was~~ heated at 45°C.~~ The LB agar was~~ and poured onto an LB agar medium plate containing 1.5% agar. After overnight ~~incubation~~culturing at 37°C, ~~HyBond-~~HYBOND® N™ (Amersham Co., Ltd.) was ~~placed to~~brought into intimate contact with the plate ~~on which~~having plaques ~~were produced and stored~~formed thereon for about 3 minutes. ~~According to a conventional method~~Then, the~~is~~ filter was ~~treated with~~subjected to an alkaline ~~solution~~denaturation treatment in accordance with a commonly used method, neutralized, and ~~dipped~~immersed in a ~~2x SSC~~X SSC solution. The DNA was ~~then immobilized onto~~fixed on the filter ~~using the~~by UV CROSSLINK~~ER~~ (Stratagene Co., Ltd.). The ~~resulting~~ obtained filter ~~was dipped into~~ was immersed in a Rapid-hyb buffer (Amersham Co.~~)~~, Ltd.~~ After pretreatment~~) and pretreated at 65°C for 15 minutes ~~at 65°C, the~~. Thereafter, the filter was ~~placed in Rapid-hyb~~ transferred into the above buffer containing the above heat- denatured human OBM cDNA fragments (about 690 bp, 5~~x~~ X 10^5 cpm/ml) ~~described above. After~~and allowed to hybridize at 65°C overnight ~~hybridization at 65°C~~. After the reaction, the filter was washed with ~~2 x SSC, 1 x SSC, and 0.1 x SSC, each~~ 0.1%-SDS-containing ~~0.1% SDS~~2X SSC once, with 1X SSC once and with 0.1X SSC once in ~~this order respectively~~turn at 65°C for 15 minutes~~ at 65°C~~. ~~Several~~The obtained positive clones ~~obtained~~ were ~~further purified by repeating~~screened two more times so as to purify the ~~screening twice~~clones. A clone ~~possessing an insert~~ ~~(~~having about 2.2 kb~~)~~ of insert was selected ~~from the purified clones~~out of these and ~~was~~ used in the following experiments. This~~e~~ purified phage was named ~~λ~~λhOBM. ~~About~~From the purified λhOBM, about 10 ~~μ~~μg of DNA was obtained ~~from the purified λ hOBM using~~in accordance with a protocol of a QIAGEN® Lambda kit (~~Qiagen~~QIAGEN Co., Ltd.)~~ according to the manufacturer's protocol~~. ~~The~~After this DNA was ~~digested~~cleaved with a restriction enzyme ~~SalI and subjected to electrophoresis~~

~~on an agarose gel to separate the~~SalI, about 2.2 kb of hOBM insert cDNA ~~(about 2.2 kb)~~was separated by agarose electrophoresis. This DNA fragment, purified ~~using the~~by use of a QIAEX® gel extraction kit (~~Qiagen~~QIAGEN Co., Ltd.), was ~~digested~~cleaved with restriction enzyme ~~SalI~~SalI in advance and then inserted into dephosphorylated plasmid pUC19 (MBI Co., Ltd.) ~~which was previously digested with a restriction enzyme SalI and dephosphorylated, using~~by use of a DNA ligation kit ver. 2 (Takara Shuzo Co., Ltd.). *Escherichia coli* DH 5-~~e~~α (Gibco BRL Co., Ltd.) was transformed ~~with~~by use of the pUC19 containing ~~the resulting~~obtained DNA fragment. The ~~resulting~~obtained transformant was named pUC19hOBM. ~~The~~After proliferating the transformant ~~was grown and pUC19hOBM in which the~~, about 2.2 kb of human -OBM cDNA ~~(about 2.2 kb) was~~ inserted ~~and~~plasmids were purified ~~by~~thereform in accordance with a ~~conventional~~commonly used method.

(5) Determination of ~~nucleotide sequence~~the Nucleotide Sequence of cDNA ~~e~~Encoding the ~~entire amino acid sequence~~Full Length Amino Acid Sequence of ~~h~~Human OBM

The nucleotide sequence of the ~~resulting~~ human OBM cDNA obtained in Example 19 -(4) was determined ~~using the~~by use of a Taq ~~Dye DeoxyDideoxy~~ Terminator Cycle Sequencing FS kit (Paerkin Elmer Co., Ltd.). ~~Specifically~~That is, the nucleotide sequence of the inserted fragment was determined ~~using~~by use of pUC19hOBM as a template. ~~As primers, primers for the determination of the nucleotide sequence of the inserted fragment DNA in pUC19hOBM,~~ M13 Primer M~~3 and~~3, M13 Primer RV (~~manufactured by Takara Shuzo Co~~TAKARA SHUZO CO., LTD.), and a synthetic primer~~,~~ human OBM #~~8,~~8 designed based on the nucleotide sequence of the human OBM cDNA fragment (about 690 bp) were used. ~~The~~ as primers for determining the nucleotide sequence of the inserted fragment DNA of pUC19. The sequences of the primers ~~used~~, M13 Primer M3 and M13 Primer RV, are shown in SEQ ID NO: 4 and SEQ ID NO: 5, respectively ~~shown as the Sequence ID No. 4 and No. 5.~~ The amino acid sequence of human OBM ~~deduced~~estimated from the nucleotide sequence of the human OBM cDNA is shown in ~~the Sequence Table as Sequence~~SEQ ID ~~No. 11. The~~NO: 11, and the nucleotide sequence of the human OBM cDNA is shown ~~as~~in ~~Sequence~~SEQ ID ~~No.~~NO: 12.

The obtained plasmid containing the human OBM cDNA and the obtained *Escherichia coli* ~~which was~~ transformed by ~~the~~ pUC19hOBM~~, which is the plasmid containing the resulting human OBM cDNA, was~~ were deposited ~~in~~with the National Institute of Bioscience and Human ~~-~~Technology~~,~~ of the Agency of Industrial Science and Technology~~, on August 13, 1997 as~~ of the Ministry of International Trade and Industry with the deposition ~~No.~~number FERM BP-~~6058.~~6058 on August 13, 1997.

[Example 20]

~~Radioiodination~~^{125}I Labeling of OCIF ~~with ^{125}I~~ and ~~a~~Quantitative ~~analysis~~ Determination of ^{125}I-~~l~~Labeled OCIF by ELISA

OCIF was ^{125}I-labeled in accordance with ~~^{125}I using the IODO-GEN~~Iodogen method. ~~Twenty~~20 ~~μ~~μl of 2.5 mg/ml ~~IODO-GEN~~Iodogen-chloroform solution was transferred to a 1.5 ml Eppendorf tube, and ~~the~~ chloroform was evaporated at 40°C~~,~~ ~~thereby providing a tube~~ so as to prepare an Iodogen-coated ~~with IODO-GEN~~tube. ~~The~~After the tube was washed ~~three times~~ with 400 ~~μ~~μl of 0.5 M sodium phosphate buffer ~~solution~~ (Na-Pi~~,~~; pH 7.0) three times~~, followed by the addition of~~ 5 ~~μ~~μl of 0.5 M Na-Pi ~~(~~with a pH of 7.0~~). To this tube~~ was added. Immediately after 1.3 ~~μ~~μl (18.5 MBq) of Na-^{125}I solution (Amersham Co., Ltd., NEZ-033H)~~, immediately followed by~~ was added to the ~~addition of~~tube, 10 ~~μ~~μl of 1 mg/ml OCIF solution (monomer type or dimmer type) was added. The ~~mixture~~resulting solution was mixed ~~in~~by means of a vo~~l~~rtex mixer and ~~allowed~~left to stand at room temperature for 30 seconds. Thi~~s~~e solution was transferred to a tube ~~to which~~containing 80 ~~μ~~μl of 0.5 M Na-Pi ~~(pH 7.0)~~ solution ~~containing~~(pH 7.0), which contained 10 mg/ml potassium iodi~~n~~de and 5 ~~μ~~μl of ~~a~~ phosphate buffered saline ~~solution~~ containing 5% bovine serum albumin (BSA-PBS) ~~were previously added~~, and mixed. The solution was ~~mixed, applied~~added to a spin column (1 ml, G-25 Sephadex® fine, ~~manufactured by~~ Pharmacia Co., Ltd.) ~~which was~~ equilibrated with BSA-PBS ~~in advance,~~ and centrifuged at 2,000 rpm for 5 minutes ~~at 2,000 rpm~~. ~~Four~~After ~~hundred~~400 ~~μ~~μl of BSA-PBS was added to ~~the~~ a fractions eluted from the column~~. After mixing~~ and the fraction was mixed, 2 ~~μ~~μl of the ~~solution~~each fraction was ~~used to measure~~sampled, and the radioactivity of the sample was measured by means of a gamma counter. The radiochemical purity of the prepared ^{125}I- labeled OCIF solution ~~obtained above~~ was ~~measured~~determined by ~~counting~~measuring the

radioactivity of ~~fractions~~a fraction precipitated by addition 10% trichloroacetic acid (TCA).

The OCIF biological activity of the ^{125}I- labeled OCIF was measured ~~according to the~~in accordance with a method described in WO 96/26217. ~~The~~Further, the concentration of ~~the~~ ^{125}I- labeled OCIF was measured ~~using the~~by ELISA ~~method as follows~~in the following manner. ~~Specifically~~That is, 100 µl of 50 mM $NaHCO_3$ (pH 9.6) ~~in which~~, having 2 µg/ml of rabbit anti-OCIF polyclonal antibody described in ~~the~~ WO 96/26217 ~~was~~ dissolved ~~to a concentration of 2 µg/ml~~therein, was added to each well of ~~a~~ 96-well immunoplate (~~MaxiSorp™, manufactured by~~ Nunc Co.) ~~in the amount of 100 µl per well. After these wells were allowed~~, Ltd., MaxiSorp) and left to stand at 4°C overnight ~~at 4°C~~;. After this solution was ~~removed. Then the wells were charged with a mixed aqueous~~ discarded, 200 µl of mix-solution of ~~Block Ace™~~BLOCKACE (Snow Brand Milk Products Co., Ltd.) and a phosphate buffered saline ~~solution~~ (mixing ratio = 25:75) (B-PBS) ~~in the amount of 200 µl/~~was added to each well. ~~The plate was~~ and then ~~allowed~~left to stand ~~for two hours~~ at room temperature for 2 hours. After the solution was ~~removed~~discarded, ~~the~~ each wells ~~were~~ was washed ~~three times~~ with a phosphate buffered saline ~~solution~~ containing 0.01% Polyso~~lv~~rbate 80 (P-PBS) three times. ~~Next~~Thereafter, 100 µl of B-PBS containing a ^{125}I- labeled OCIF sample or ~~the~~OCIF reference standard ~~OCIF~~ was added ~~in the amount of 100 µl/~~to each well. ~~The plate was then allowed~~ and left to stand ~~for two hours~~ at room temperature for 2 hours. After the solution was ~~removed~~discarded, each well was washed ~~six times~~ with 200 ~~µ~~µl of P-PBS six times. ~~A~~Then, 100 µl of diluted solution ~~prepared by diluting~~of peroxidase-labeled ~~rabbit~~ anti-OCIF rabbit polyclonal antibody ~~with~~in B-PBS was added ~~in the amount of 100 µl/~~to each well. ~~The plate was allowed~~ and left to stand ~~for two hours~~ at room temperature for 2 hours. After the solution was ~~removed~~discarded, ~~the~~ each wells ~~were~~ was washed ~~six times~~ with 200 ~~µ~~µl of P-PBS six times. Then, ~~a~~100 µl of TMB solution (TMB Soluble Reagent, High Sensitivity, Scytek Co., Ltd.) was added ~~in the amount of 100 µl/~~to each well. ~~After being allowed~~ and then left to stand at room temperature for 2- to 3 minutes. Thereafter, 100 ~~µ~~µl of ~~a termination solution~~ (Stopping Reagent~~,~~ (Scytek Co., Ltd.) was added to each well. ~~Absorbance~~The absorbance of each well at 450 nm was measured ~~at 450 nm using~~ by means of a microplate reader. The

concentration of the ^{125}I- labeled OCIF was determined ~~with~~from a calibration curve prepared ~~using~~by use of the OCIF reference standard ~~OCIF~~.

~~<~~[Example 21~~>~~]

Expression of ~~the protein encoded~~Protein Encoded by the cDNA of the ~~p~~Present ~~i~~Invention

(1) Construction of hOBM ~~e~~Expression ~~v~~Vector for ~~a~~Animal ~~c~~Cells

pUChOBM was ~~digested~~cleaved with restriction enzyme ~~SalI~~SalI, and ~~a DNA fragment (~~about 2.2 kb~~) were~~ DNA fragments were purified by 1% agarose gel electrophoresis ~~on an 1% agarose gel. The ends of the DNA fragments were blunted using a~~and blunt-ended with DNA ~~b~~Blunting ~~k~~Kit (Takara Shuzo Co., Ltd.) (~~blunted hOBMcDNA~~the resulting DNA fragment with smoothed terminals is called "smoothed hOBM cDNA fragment"). Expression plasmid pcDL-SR ~~α~~α296 (Molecular and Cellular Biology, Vol. 8, pp. 466~~-~~ to 472 (1988)) was ~~digested~~cleaved with a restriction enzyme EcoRI, ~~blunted with~~and blunt-ended with the blunting kit ~~and ligated with the blunted hoBM~~. The resulted expression plasmid was bound to the smoothed hOBM cDNA fragment ~~using~~by use of a DNA ligation kit ver. 2. Using the ligation reaction solution, *Escherichia coli* DH-~~α~~α was transformed ~~with the ligation reaction. A plasmid in~~. From the ~~resulting~~obtained ampicillin -resistant transformant ~~was subjected to digestion with restriction enzyme to analyze the DNA restriction map and determine the DNA sequence. As a result~~, a ~~strain~~clone, having a phOBM plasmid in which hOBM cDNA ~~is~~ inserted ~~in the same~~with forward direction ~~of~~for transcription ~~as that~~direction of SR-~~α promoter~~α promoter, was selected by analysis of DNA map obtained by restriction enzyme cleavage and determination of DNA sequences. The ~~microorganism~~obtained ~~strain~~clone was named DH5-~~α~~α/phOBM.

(2) Expression of ~~h~~Human OBM in COS-7 ~~c~~Cells

~~Escherichia~~*E coli*, DH5-~~α~~α/phOBM, was cultured and the plasmid phOBM was purified ~~using Qiafilter~~with QIA® Filter Plasmid Midi ~~k~~Kit (~~Qiagen~~QIAGEN Co., Ltd.). The phOBM was transfected ~~using Lipofectamine~~ into COS-7 cells in ~~the~~each wells of ~~a~~ 6- well plate by use of lipofectamine, and the cells were cultured ~~for two days~~ in DMEM containing 10% fetal bovine serum for 2 days. The ~~culture~~ medium was replaced with cysteine-/methionine-free DMEM (~~manufactured by~~ Dainippon ~~Seiyaku~~Pharmaceutical

Co., Ltd.) ~~to which~~containing 5% dialyszed fetal bovine serum ~~was added~~ (88 ~~μ~~μl/well)~~. The~~, and the cells were ~~incubated~~cultured for another 15 minutes. Then, ~~followed by addition of~~ 14 ~~μ~~μl of Express Protein Labeling Mix (NEN Co., Ltd., 10 mCi/ml) was added. After ~~four~~the cells were cultured for 4 hours ~~incubation~~, 200 ~~μ~~μl of DMEM containing 10% fetal bovine serum was added ~~to each well. The~~, and the cells were cultured for ~~one~~1 hour ~~and~~. After the cells were washed ~~twice~~ with PBS~~. Then~~ twice, 0.5 ml of ~~a~~ TSA buffer (10 mM Tris-HCl (pH 8.0) containing 0.14 M NaCl and 0.025% NaN_3~~, pH 8.0~~) containing 1% Triton X-100, 1% bovine hemoglobin, 10 ~~μ~~μg/ml leupeptin, 0.2 ~~TIU~~TI U/ml aprotinin~~,~~ and 1 mM PMSF was added ~~to each well~~, and the ~~mixtures~~cells were ~~allowed~~left to stand ~~for one hour~~ on ice for 1 hour. ~~The~~After the cells were ~~mixed~~crushed by pipetting ~~and~~, the resulting lysate was centrifuged at 4°C and 3,000 ~~x~~X g~~,~~ for 10 minutes ~~at~~so ~~4°C,~~as to obtain ~~supernatants~~a supernatant. ~~Two hundred~~200 ~~μ~~μl of ~~a~~ dilution buffer (TSA buffer containing 0.1% Triton X-100, 0.1% bovine hemoglobin, 10 ~~μ~~μg/ml leupeptin, 0.2 TIU/ml aprotinin~~,~~ and 1 mM PMSF) was added to 100 ~~μ~~μl of the supernatant ~~from each well. The~~, and the resulting ~~mixtures were agitated at 4°C for one hour~~supernatant was shaken together with Protein A Sepharose® (50 ~~μ~~μl) ~~and~~at 4°C for 1 hour. Thereafter, the solution was centrifuged at 4°C, 1,500 ~~x~~X g for ~~one~~1 minute ~~at~~so ~~4°C,~~as to collect ~~supernatants~~a supernatant. Thereby, ~~thereby removing the~~a protein ~~which~~ non-specifically ~~adsorbed~~binding the Protein A Sepharose® was removed. OCIF (1 ~~μ~~μg) was added to the ~~supernatants~~supernatant, and the ~~mixtures were agitated~~resulting supernatant was shaken at 4°C for ~~one~~1 hour ~~at~~so ~~4°C~~as to bind ~~human~~ OBM and OCIF together. Then, ~~rabbit~~an anti-OCIF rabbit polyclonal antibody (50 ~~μ~~μg) was added, ~~followed by agitation~~and the resulting solution was shaken at 4°C for ~~one~~1 hour. Then, Protein A Sepharose® (10 ~~μ~~μl) was added to the ~~resulting~~ solution~~, followed by agitation~~ and the solution was then shaken at 4°C for ~~an additional~~1 hour. The ~~mixtures thus obtained were~~solution was centrifuged at 4°C, 1,500 X g for 1 minute ~~at 1, 500 x g at 4°C~~so as to collect ~~precipitates~~a precipitated fraction. The ~~precipitates were~~precipitate resulting from the centrifugation was washed ~~twice~~ with ~~a~~the dilution buffer twice, ~~twice~~ with a bovine hemoglobin- free dilution buffer twice, ~~once~~ with a TSA buffer once, and ~~once~~ with 50 mM Tris-HCl (pH 6.5) once. After ~~addition~~washing,

~~of~~an SDS buffer ~~containing 10% β-mercaptoethanol~~ (0.125 M Tris-HCl, 4% dodecyl sodium ~~dodecylsulfate~~sulfate, 20% glycerol, 0.002% ~~B~~bromophenol ~~B~~blue, pH 6.8)~~, the mixture~~ containing 10% β-mercaptoethanol was added to the precipitate. The precipitate was heated at 100°C for 5 minutes ~~at 100°C~~, and it was subjected to SDS-PAGE (12.5% polyacrylamide gel, Daiichi ~~Pure Chemical~~Kagaku Co., Ltd.). The gel was fixed and dried ~~according to~~in accordance with a ~~conventional~~commonly used method~~. After isotope~~, and the signals of isotopes from the fixed gel were ~~enhanced~~amplified ~~using~~by Amplify™® (Amersham Co.~~)~~, ~~the dried~~Ltd.). The fixed gel was ~~subjected~~exposed to ~~autoradiography at -80°C using Bio-Max~~BioMax® MR ~~f~~Film (Kodak Co., Ltd.) at -80°C. The results are shown in ~~Figure 18, which shows~~Fig. 8. As a result, it was revealed that the molecular weight of ~~the~~ protein encoded by the cDNA of the present invention ~~is~~was about 40,000.

~~<~~[Example 22~~>~~]

Binding of ~~the protein encoded~~Protein Encoded by the cDNA of the ~~p~~Present ~~i~~Invention ~~and~~ to OCIF

~~PhOBM, which was purified in~~In the same manner as in ~~the~~ Example 21-(2), the purified phOBM was transfected into COS-7 cells in each well of a 24- well plate ~~using Lipofectamine. After~~by the use of lipofectamine, and the cells were cultured for 2 ~~to~~or 3 days. Then, the cells were washed with serum-free DMEM~~. Two hundred~~, and 200 ~~μ~~μl of ~~a culture~~ medium for ~~a~~the binding ~~test medium~~assay (serum-free DMEM ~~to which~~containing 0.2% bovine serum albumin, 20 mM Hepes buffer ~~solution~~, 0.1 mg/ml heparin~~,~~ and 0.2% NaN_3 ~~were added~~), containing 20 ng/ml of ^{125}I- labeled OCIF was added to ~~the~~some wells. ~~To the~~In addition, to other wells, 200 ~~μ~~μl of ~~culture medium for the binding test medium~~the medium for binding assay, containing 8 ~~μ~~μg/ml of unlabeled OCIF in addition to 20 ng/ml of the ^{125}I- labeled OCIF, was added~~. After incubation for one hour at 37°C in a~~ so as to conduct following experiments. After culture in a CO_2 incubator (5% CO_2)~~, the cells were washed twice with 500 μl of a phosphate buffered saline solution containing 0.1 mg/ml of heparin. Then, 500 μl of 0.1 N NaOH solution was added to each well and the plate was allowed~~ at 37°C for 1 hour, the cells were washed twice with 500 μl of phosphate buffered saline containing 0.1 mg/ml heparin. After washing, 500 μl of 0.1 N NaOH solution was added to each well, and the wells

were then left to stand ~~for 10 minutes~~ at room temperature ~~to dissolve the~~ for 10 minutes so as to dissolve the cells~~.~~. The ~~radioactivity~~amount of ^{125}I ~~in the wells was measured by a gamma counter. As a result, as shown in Figure 19,~~in each well was measured by means of a gamma counter. As a result, it was confirmed that the ^{125}I- labeled OCIF bounds only to ~~the~~ a cells transfected with phOBM~~. Moreover,~~ as shown in Fig. 19. Further, it was also confirmed that the binding ~~was significantly inhibited by adding 400-fold excess unlabelled OCIF (8 μg/ml). Based on the results described above, the protein, human OBM encoded by the cDNA in the phOBM was confirmed to specifically bind to OCIF on the surface of~~ was significantly inhibited by addition of a 400-fold concentration of unlabeled OCIF (8 μg/ml). From these results, it was revealed that a human OBM protein, coded for by a cDNA on phOBM, specifically bound to OCIF on the surface of a COS~~-~~7 cells~~.~~

~~<~~

[Example ~~23>~~
23]

Crosslinking ~~of ^{125}I-labeled OCIF and~~Experiment of ^{125}I Labeled OCIF to Protein Encoded by the cDNA of the Present Invention

To further analyze the characteristics of the protein encoded by the cDNA of the present invention, crosslinking of ^{125}I labeled monomer type OCIF with the protein encoded by the cDNA of the present invention~~Crosslinking of ^{125}I-labeled monomer type OCIF and the protein encoded by the cDNA of the present invention was carried out to further investigate the characteristics of the protein encoded by the cDNA of the present invention. After constructing expression vector phOBM and transfecting into COS-7 cells according to the method used in the~~ was conducted. That is, after expression vectors phOBM were prepared and transfected into COS-7 cells in accordance with the methods described in Examples 21-(~~1~~)1) and ~~21~~(2), ~~200~~ 200 μμl of ~~binding test medium~~ the medium for the binding assay containing the ^{125}I- labeled OCIF (25 ng/ml) was added to some wells. In addition, the medium for the binding assay, containing unlabeled OCIF of a 400-fold concentration in addition to the ^{125}I labeled OCIF ~~(25 ng/ml) described above,~~ was added~~. The binding test medium~~ to ~~which unlabeled OCIF was added at a~~

~~400-fold concentration in addition to ^{125}I-labeled OCIF was used for the other wells. After cultured for one hour at 37°C in a~~ other wells. The cells were cultured in a CO_2 incubator (5% CO_2)~~, the cells were washed twice with 500 µl of phosphate buffered saline containing 0.1 mg/ml heparin. Five hundred µl of phosphate buffered saline in which~~ at 37°C for 1 hour, and the cells were washed twice with 500 µl of phosphate buffered saline containing 0.1 mg/ml of heparin. To these cells, 500 µl of phosphate buffered saline containing 100 ~~µ~~µg/ml of ~~a~~ crosslinking agent (~~D~~DSS: (~~d~~Disuccinimidyl suberate, ~~manufactured by~~ Pierce Co., Ltd.) was ~~dissolved was~~ added ~~to~~, and the cells~~, followed by incubation~~ incubated at 0°C for 10 minutes ~~at~~for ~~0°C~~reaction. ~~The~~After the cells in these wells were washed twice with 1 ml of ~~ice-cold~~ phosphate buffered saline~~. After an addition of~~ cooled to 0°C, 100 ~~µ~~µl of 20 mM Hepes buffer ~~solution~~ containing 1% Triton X-100 (Wako Pure ~~Chemicals Co.~~Chemical Industries, Ltd.), 2 mM PMSF (~~P~~phenylmethylsulfonyl fluoride, Sigma Co., Ltd.), 10 ~~µ~~µM ~~P~~pepstatin (Wako Pure Chemicals ~~Co.~~Industries, Ltd.), 10 ~~µ~~µM leupeptin (Wako Pure ~~Chemicals Co.~~Chemical Industries, Ltd.), 10 ~~µ~~µM antipain (Wako Pure ~~Chemicals Co.~~Chemical Industries, Ltd.) and 2 mM EDTA (Wako Pure Chemicals ~~Co.~~Industries, Ltd.) were added to these cells, and the wells were ~~allowed~~left to stand at room temperature for 30 ~~minutes at room temperature~~minute so as to ~~dissolve~~lyse the cells. ~~These~~After 15 µl of these samples ~~(15 µl aliquots)~~ were treated with SDS under ~~reducing~~nonreducing conditions ~~according to~~in accordance with a ~~conventional~~commonly used method ~~and~~, the samples were subjected to electrophoresis with a gel for SDS- electrophoresis ~~using~~ (4- to 20% polyacrylamide gradient ~~gel~~ (, Daiichi ~~Pure Chemical~~Kagaku Co., Ltd.). After the electrophoresis, the gel was dried and ~~subjected~~exposed to ~~autoradiography for 24 hours at -80°C using~~ BioMax® MS ~~film~~Film (Kodak Co., Ltd.) with BioMax® MS Intensifying Amplifying Screen (Kodak ~~Co.~~Co., Ltd.) ~~and BioMax MS sensitization screen (Kodak Co.)~~ at -80°C for 24 hours. The ~~film subjected to autoradiography was~~exposed films were developed ~~according to~~in accordance with a ~~conventional~~commonly used method. As a result, a protein band ~~of~~having a molecular weight ~~in the range~~ of about 90,000 ~~110,000, shown in Figure 20,~~ to 110,000 was detected as shown in Fig. 20 by crosslinking ~~of~~between ^{125}I-labeled monomer type OCIF and the protein encoded by the cDNA of the present invention.

<[Example 24>]

Expression of sSecretedory-form-Type hHuman OBM

(1) Construction of sSecretedory-formType hHuman OBM eExpressiong pPlasmid

A PCR reaction was carried out usingby use of human OBM SF (Sequence Table, SequenceSEQ ID No.NO: 13) and mouse OBM #8 (Sequence Table, SequenceSEQ ID No.NO: 6) as primers and pUC19hOBM as a template. After the product was purified by agarose gel electrophoresis on an agarose gel, the product was digested, it was cleaved with restriction enzymes SP1HSpI and HindIII, and furtherthen purified by agarose gel electrophoresis on an agarose gelso as to obtain a purified fragment (0.27 kb) fragment. Human OBMA fragment of hOBM cDNA which was partially digested withcleaved at only one site of restriction enzyme DraI by partial cleavage of human OBM cDNA therewith, and DNA fragments digested with DraI at one site were purified by agarose gel electrophoresis on an agarose gel. The, and the purified fragment was further digestedcleaved with a restriction enzyme HindIII. The 0.53 kb of DraI/HindIII fragment was purified by agarose gel electrophoresis on an agarose gel. The, and the purified fragment was ligated withand the 0.27 kb Sp1HSpI/HindIII fragment derived from fragment (0.27 kb) of the PCR described above using ligation kit ver. 2 (Takara Shuzo Co.)PCR product together with HindIIIan SpI/EcoRIV fragment (5.2 kb) of pSec TagA (InvirogenInvitrogen Co.), Ltd.) were subjected to ligation by use of a ligation kit ver. 2 (TAKARA SHUZO CO., LTD.), and *Escherichia coli* DH5-α wasα were transformed usingby use of the reaction product of ligation. Plasmids were purified by alkali SDS method from the resultingobtained ampicillin -resistant transformants and digested withclone by alkaline SDS method and cleaved by restriction enzymes so as to select a plasmid containinghaving 0.27 kb and 0.53 kb- of fragments as insertsinserted in pSec TagA. Thise plasmid was confirmedsubjected to have a sequence encoding the secreted human OBM by sequencing using Tag dyedeoxyterminator cycle sequencing by use of a Taq Dideoxy Terminator Cycle Sequencing FS kKit (Perkin Elmer Co., Ltd.), thereby confirming that the plasmid had sequences encoding secretory-type human OBM. TheAfter the plasmid was digestedcleaved withby restriction enzymes NhelI and XhoI to prepareXhoI, a fragment (0.8 kb) corresponding to the secretedsecretory-type human OBM cDNA was collected by agarose gel electrophoresis on an agarose gel. This

fragment was inserted into ~~the~~an NheI ~~and~~ /XhoI fragment (10.4 kb) of an expression vector pCEP4 (Invitrogen Co., Ltd.) ~~using~~by use of the ligation kit, and *Escherichia coli* DH5~~-α was~~α were transformed ~~using~~by use of the reaction product of the ligation. Plasmids were purified ~~by alkali-SDS method~~ from the ~~resulting~~obtained ampicillin - resistant ~~transformants~~clones by alkaline SDS method and ~~digested~~cleaved ~~with~~by restriction enzymes so as to select ~~an~~a *Escherichia coli* clone having ~~the~~a secretory-type human OBM expression plasmid ~~for secreted-form human OBM~~ (pCEPshOBM) with a target structure. The *Escherichia coli* ~~containing~~clone having the pCEPshOBM was cultured, and the pCEPshOBM was purified ~~using Qiafilter plasmid midi kit~~by use of QIA® Filter Plasmid Midi Kit (~~Qiagen~~QIAGEN Co., Ltd.).

(2) Expression of ~~s~~Secreted~~ory-form~~Type OBM

293-EBNA cells were suspended in IMDM containing 10% FCS (IMDM-10% FCS), ~~added into a~~seeded in a collagen-coated 24- well plate ~~coated with collagen~~ (~~manufactured by~~ Sumitomo Bakelite Co., Ltd.) in ~~a cell density~~an amount of 2~~×~~ X 10^5 cells/2 ml/well, and cultured overnight. ~~The~~To the cells ~~were transfected with~~, 1 ~~μ~~μg of pCEPshOBM or pCEP4 ~~using~~was transfected by use of 4 ~~μ~~μl of ~~L~~lipofectamine (Gibco Co.~~)~~, Ltd. ~~After~~), and the cells were cultured for ~~two~~another 2 days in 0.5 ml of ~~a~~ serum-free IMDM or IMDM-10% FCS, ~~the culture supernatants were collected~~thereby collecting a conditioned medium. Expression of ~~the secreted~~secretory-type human OBM in the ~~culture~~conditioned ~~supernatant~~medium was ~~detected as follows~~confirmed in the following manner. ~~Sodium bicarbonate~~That is, sodium hydrogen carbonate was added to the ~~culture~~conditioned ~~supernatants~~medium to a final concentration of 0.1 M and ~~the mixtures were added to a 96-well plate. The plate was allowed~~left to stand at 4°C overnight ~~at 4°C~~, ~~thereby~~and ~~immobilizing~~the human OBM in the ~~culture supernatants on the~~conditioned medium was solid-phased in a 96- well plate. ~~The plate was blocked using Block Ace™~~BLOCKACE (Snow Brand Milk Products Co., Ltd.) solution ~~four-fold~~ diluted 4 times with PBS (B-PBS) was added to each well and ~~allowed~~the plate was left to stand ~~for two hours~~ at room temperature for 2 hours to cause blocking. ~~After adding~~ 3-100 ng/ml of OCIF ~~which was~~ diluted with B-PBS was added to ~~each well,~~ the ~~plate was allowed~~wells and left to stand at 37°C for ~~two~~2 hours ~~at 37°C, followed by wash~~. After the plate was washed with PBS containing 0.05% Polyso~~lv~~rbate 20 (P-PBS)~~.~~

~~Then~~, 100 ~~μ~~μl of ~~a~~ peroxidase- labeled ~~rabbit~~ anti-OCIF ~~polyclonal~~ antibody described in WO 96/26217 ~~which was~~ diluted with B-PBS was added to each well~~. After allowing~~ and left to stand at 37°C for ~~two~~2 hours ~~at 37°C, the wells were~~. After each well was washed ~~six times~~ with P-PBS~~. Then~~ six times, 100 μl of TMB solution (TMB Soluble Reagent, High Sensitivity, Scytek Co., Ltd.) was added ~~in the amount of 100 μl per~~to each well and ~~the mixture was allowed~~then left to stand at room temperature for about 10 minutes. ~~The reaction was terminated by the addition of~~Thereafter, 100 ~~μ~~μl of ~~termination solution~~ (Stopping Reagent~~,~~ (Scytek Co., Ltd.) was added to each well. ~~Absorbance~~The absorbance of each well at 450 nm ~~for each well~~ was measured by means of a microplate reader. The results are shown in ~~Figure 21, which indicates that the absorbance at 450 nm increased according to the concentration of the added OCIF in~~Fig. 21. In the plate ~~in which~~having the solid-phased conditioned medium of the cells transfected with the pCEPshOBM ~~was immobilized.~~, absorption at 450 nm increased depending on the concentration of the OCIF added. ~~On~~ Meanwhile, in the case where the conditioned medium of the ~~other hand, no increase in absorbance was seen in the wells in which the conditioned medium of the cells~~cells transfected ~~with~~only with the vector pCEP4 was ~~immobilized.~~solid-phased, no increase in absorption was seen. Further, ~~Figure~~Fig. 22 shows the results of an experiment ~~wherein~~in which the proportion of the conditioned medium used for ~~immobilization~~solid phasing was ~~changed~~varied within a range of 5- to 90% ~~in the presence of~~and a ~~con~~certain~~t~~ concentration of OCIF (50 ng/ml) was added. ~~The absorbance~~In the plate having the solid-phased conditioned medium of the cells transfected with the pCEPshOBM, absorption at 450 nm increased ~~according to the~~along with an increase in the proportion of the conditioned medium ~~in the plate wherein the conditioned medium of the cells transfected with pCEPshOBM was immobilized~~added. Meanwhile, ~~whereas no such increase in absorbance was seen~~ in the plate ~~wherein~~having the solid-phased conditioned medium of the cells transfected with the vector pCEP44, no increase in absorption was ~~immobilized~~observed. From these results, it was confirmed that secrete~~d~~ory ~~form~~type human OBM ~~is~~was ~~produced~~expressed in the conditioned medium of the cells transfected with ~~pCBPshOBM~~the pCEPshOBM.

~~<~~[Example 25~~>~~]

Expression of ~~t~~Thioredoxin~~-h~~Human OBM ~~f~~Fusion ~~p~~Protein (Trx-hOBM)

(1) Construction of ~~a thioredoxin~~Thioredoxin-~~h~~Human OBM ~~f~~Fusion ~~p~~Protein (Trx-hOBM) ~~e~~Expression ~~v~~Vector

~~Ten~~10 ~~μ~~μl of 10X ExTaq buffer (Takara Shuzo Co., Ltd.), 8 ~~μ~~μl of 10 mM dNTP (Takara Shuzo Co., Ltd.), 77.5 ~~μ~~μl of sterilized distilled water, 2 ~~μ~~μl of ~~an~~pUC19hOBM aqueous solution ~~of pUC19hOBM~~ (10 ng/~~μ~~μl), 1 ~~μ~~μl of primer~~,~~ mouse OBM #3 (SEQ ID NO: 9) (100 pmol/~~μ~~μl~~, Sequence Table, Sequence ID No. 9)~~), 1 ~~μ~~μl of primer~~,~~ hOBM SalR2 (SEQ ID NO: 14) (100 pmol/~~μ~~μl~~, Sequence Table, Sequence ID No. 14),~~) and 0.5 ~~μ~~μl of ExTaq (5 ~~u~~μ/~~μ~~μl) (Takara Shuzo Co., Ltd.) were mixed ~~and reacted (PCR)~~together in a ~~micro centrifuge~~microcentrifuge tube so as to cause a PCR reaction. After the reaction ~~at~~consisiting of 95°C for 5 minutes, ~~at~~ 50°C for ~~one~~1 second, ~~at~~ 55°C for ~~one~~1 minute, ~~at~~ 74°C for ~~one~~1 second~~,~~ and ~~at~~ 72°C for 5 minutes, ~~a~~the cycle reaction consisting of ~~a reaction~~ at 96°C for ~~one~~1 minute, ~~at~~ 50°C for ~~one~~1 second, ~~at~~ 55°C for ~~one~~1 minute, ~~at~~ 74°C for ~~one~~1 second~~,~~ and ~~at~~ 72°C for 3 minutes, was repeated 25 times. ~~From the total reaction mixture~~An approximately 750 bp DNA fragment ~~(750 bp)~~ was purified. ~~The~~ from the whole ~~amount of~~reaction solution. After the purified DNA fragment (whole) was ~~digested~~cleaved with restriction enzymes SalI (~~Takara Shuzo Co~~TAKARA SHUZO CO., LTD.) and BspHI (~~New England Bilabs Co~~NEW ENGLAND BILABS CO., LTD.), ~~and subjected to electrophoresis on a~~ 1% agarose gel electrophoretic migration was carried out so as to ~~obtain purified~~purify an approximately 320 bp DNA fragment (~~Fragment 1, about 320 bp). The~~ fragment ~~was dissolved~~ 1) and dissolve the fragment in 20 ~~μ~~μl of sterilized distilled water. ~~In the same manner,~~ Similarly, an approximately 450 bp DNA fragment (~~F~~fragment ~~2, about 450 bp) obtained by digesting~~ 2) which is a cleaved product of 4 ~~μ~~μg of pUC19hOBM ~~with~~described in Example 19-(3) by a restriction enzymes BamHI~~,~~ and BspHI (~~Takara Shuzo Co~~TAKARA SHUZO CO., LTD.) and about 3.6 kb of DNA fragment (~~Fragment 3, about 3.6 kb~~fragment 3~~), obtained by digesting~~ which is a cleaved product of 2 ~~μ~~μg of pTrXFus (In~~V~~vitrogen Co., Ltd.) ~~with~~by a restriction enzymes BamHI~~,~~ and SalI (~~Takara Shuzo Co~~TAKARA SHUZO CO., LTD.) were ~~respectively~~ purified and then dissolved in 20 ~~μ~~μl of sterilized distilled water. ~~The QIAEXII~~To purify the DNA fragments, a QIAEXR

II gel extraction kit was used ~~for purification of the DNA fragments~~. ~~Fragments 1~~ Fragment 1, 2 and 3 were ~~ligated~~combined by ~~incubating at 16°C for 2.5 hours using~~use of a DNA ligation kit ver. 2 (~~Takara Shuzo Co~~TAKARA SHUZO CO., LTD.) by keeping them at 16°C for 2.5 hours. ~~Using the ligation reaction,~~ *Escherichia coli* GI724 strain (Invitrogen Co., Ltd.) was transformed ~~according to the~~using the ligation reaction solution, in accordance with a method described in ~~the Instruction Manual of~~an instruction manual attached to a ThioFusion Expression System (Invitrogen Co., Ltd.). ~~A microorganism strain with~~From the obtained ampicillin-resistant transformants, a clone, having a plasmid in which ~~the~~an hOBM cDNA fragment ~~is fused in frame~~was bound to a thioredoxin gene in the same reading frame, was selected ~~from the resulting ampicillin resistant transformants~~ by analysis of DNA ~~restriction map~~mapping obtained by ~~digestion with~~ restriction enzyme cleavage and ~~by~~ determination of DNA sequences. The ~~microorganism strain thus~~ obtained strain was named GI724/pTrxhOBM~~.~~25.

(2) Expression of Trx-~~hOBM~~OBM in *Escherichia coli*

A GI724/pTrxhOBM strain and a GI724 ~~containing~~strain transformed with pTrxFus (GI724/pTrxFus) were ~~respectively~~ cultured ~~six hours with~~ shaking at ~~30~~37°C for 6 hours in 2 ml of RMG-Amp medium (0.6% Na_2HPO_4, 0.3% KH_2PO_4, 0.05% NaCl, 0.1% NH_4Cl, 2% casamino acid, 1% glycerol, 1 mM $MgCl_2$, 100 ~~μ~~μg/ml ampicillin, pH 7.4). ~~The broth (~~0.5 ml~~)~~ of the culture suspension was added to 50 ml of Induction medium (0.6% Na_2HPO_4, 0.3% KH_2PO_4, 0.05% NaCl, 0.1% NH_4Cl, 0.2% casamino acid, 0.5% glucose, 1 mM $MgCl_2$, 100 ~~μ~~μg/ml ampicillin, pH 7.4) and cultured ~~with~~ shaking at 30°C. ~~When OD_{600nm} reached about 0.5,~~ L-tryptophan was added so as to achieve a final concentration of 0.1 mg/ml~~, followed by culturing with~~ when the value at $OD_{600\ nm}$ became about 0.5, and the cells were further shaking-cultured at 30°C for ~~an additional~~another 6 hours. The cculture ~~broth~~suspension was centrifuged at ~~3000~~3,000 ~~×~~X g so as to collect ~~the~~ cells, ~~which were~~and then the collected cell was suspended in 12.5 ml of PBS. The suspension was subjected to an ultrasonic generator (Ultrasonics Co., Ltd.) so as to ~~disrupt~~crush the cells. Then ~~disrupt cells were~~ the sample was centrifuged at ~~7000~~7,000 ~~×~~X g for 30 minutes so as to ~~obtain a supernatant liquid as a~~ collect a soluble protein fraction as a supernatant. ~~Ten~~10 ~~μ~~μl of ~~this soluble protein~~the solution fraction was subjected to SDS-PAGE (10% polyacrylamide ~~(10%)~~

~~electrophoresis~~ under reducing conditions. As a result, ~~as~~ shown in ~~Figure~~Fig. 23, a protein band ~~with~~having a molecular weight of ~~40,000~~about 40,000, which ~~was~~could not be seen in the soluble protein fraction of GI724/pTrxFus, was detected in the soluble protein fraction of GI724/~~pTrxFus was found in the soluble protein fraction of GI724/pTrxhOBM~~pTrxOBM. ~~Accordingly~~From the above results, it was confirmed that a thioredoxin-human OBM fusion protein (Trx-~~hOBM) of thioredoxin and human~~ OBM) was expressed in the Echerichia coli clone.

(3) Binding Ability of Trx-hOBM *Escherichia coli*~~.(3) Binding capability of Trx-hOBM~~ to OCIF

~~Binding of the expressed Trx-hOBM to OCIF~~It was confirmed ~~according to~~by the following experiment that the expressed Trx-hOBM bound to OCIF. ~~Anti~~That is, 100 μl of anti-thioredoxin antibody (Invitrogen Co., Ltd.) ~~which was~~ diluted ~~5000-fold~~to be 1/5,000 with 10 mM sodium hydrogen carbonate aqueous solution was added to each well of a 96- well immunoplate (Nunc Co., Ltd.), ~~in~~and the ~~amount of 100 μl per well. After being allowed~~plate was left to stand at 4°C overnight ~~at 4°C~~. After the ~~liquid~~solution in ~~the~~each ~~wells~~cell was discarded. ~~Two, hundred~~200 ~~μ~~μl of a solution ~~prepared~~obtained by diluting ~~Block Ace™~~BLOCKACE (Snow Brand Milk Products Co., Ltd.) ~~two-fold~~to be 1/2 with PBS (BA-PBS) was added to each well. ~~After being allowed~~, and then the plate was left to stand ~~for one hour~~ at room temperature~~,~~ for 1 hour. After the ~~wells were~~solution was discarded, each well was washed with P-PBS three times ~~with P-PBS~~. ~~The~~100 μl of the GI724/pTrxOBM-derived soluble protein ~~fractions originating from~~fraction solution diluted stepwise with BA-PBS, and 100 μl of the ~~above-described GI724/pTrxhOBM or~~ GI724/pTrxFus~~, each~~-derived soluble protein fraction solution diluted stepwise with BA-PBS ~~in various concentrations~~, were added to each well ~~in~~and the ~~amount of 100 μl. After being allowed~~plate was left to stand ~~for two hours~~ at room temperature~~,~~ for 2 hours. After each well was washed with P-PBS three times ~~with P-PBS and charged with~~, 100 ~~μ~~μl of OCIF (100 ng/ml) ~~which was~~ diluted with BA-PBS. ~~After being allowed~~, was added to each well and the plate was left to stand ~~for two hours~~ at room temperature~~,~~ for 2 hours. After each well was washed with P-PBS three times ~~with P-PBS and charged with~~, 100 ~~μ~~μl of peroxidase-labeled anti-OCIF antibody (described in WO 96/~~26217) which was~~26217, diluted to be 1/2,000 ~~fold~~ with BA-PBS.

~~After being allowed~~, was added to each well, and the plate was left to stand ~~for two hours~~ at room temperature~~,~~ for 2 hours. After each well was washed ~~six times~~ with P-PBS ~~and charged with~~six times, 100 ~~μ~~μl of TMB solution~~. After being allowed~~ was added to each well, and then the plate was left to stand at room temperature for about 10 minutes ~~at room temperature~~. Thereafter, ~~each well was charged with~~ 100 ~~μ~~μl of ~~termination solution (~~Stopping Reagent~~). Absorbance~~ was added to each well. The absorbance of each well at 450 nm was measured by means of a microplate reader. The results are shown in ~~Figure~~Fig. 24. ~~There was no~~No difference ~~in the absorbance~~was observed between absorbance resulted in the ~~sample with~~presence and absence of the ~~soluble protein fraction originating from~~ GI724/pTrxFus ~~added thereto~~-derived soluble protein fraction solution and, while with the ~~sample without the addition of this~~GI724/pTrxhOBM-derived soluble protein fraction~~. On the other hand~~ solution, the absorbance increased depending on an increase in ~~the samples to which the soluble protein fraction originating from GI724/pTrxhOBM was added in proportion to~~ the concentration of the GI724/pTrxOBM derived soluble protein fraction solution. ~~The~~Further, Fig. 25 shows the results of ~~the other~~an experiment ~~wherein~~in which the dilution rate of the soluble protein fraction solution to be added was ~~maintained~~kept constant (1% concentration) ~~while adding~~and OCIF (0-100 ng/ml) diluted stepwise with BA-PBS ~~in different concentrations (0-100 ng/ml) are shown in Figure 25.~~was added. ~~It can be seen that~~For the GI724/pTrxFus-derived soluble protein fraction solution, absorbance remained low ~~at any concentrations~~regardless of the concentration of OCIF ~~in samples using a soluble protein fraction originating from GI724/pTrxFus~~, ~~whereas~~while for the GI724/pTrxhOBM-derived soluble protein fraction solution, absorbance increased in ~~proportion to the~~ OCIF ~~concentration in the samples to which the soluble protein fraction originating from GI724/pTrxhOBM was added~~ concentration-dependent manner. ~~Based on these results, it~~It was confirmed from this result that Trx-hOBM ~~which is~~ produced ~~from~~in GI724/pTrxhOBM ~~has a capability of binding~~had an ability to bind OCIF.

(4) Large- sScale ~~cultivation~~ Culture of *Escherichia coli* ~~which produces~~Producing Trx-hOBM

GI724/pTrxhOBM ~~cells were~~was spread on an RMG-Amp agar medium (0.6% Na_2HPO_4, 0.3% KH_2PO_4, 0.05% NaCl, 0.1% NH_4Cl, 2% casamino acid, 1.5% agar, pH 7.4) ~~using~~with a platinum ~~transfer 100p. The cells were~~loop and cultured at 30°C overnight ~~at 30°C~~. The ~~cultured~~ cells were suspended in 10 ml of Induction medium. ~~The~~, and every 5 ml of the suspension was added ~~(5 ml for~~to each~~)~~ ~~to~~of two ~~2-1 Erlenmeyer~~conical flasks of 2L volume containing 500 ml of Induction medium, and the flasks were shaking-cultured at 30°C ~~with shaking~~. ~~When the OD_{600nm} reached about 0.5,~~ L-tryptophan was added so as to achieve a final concentration of 0.1 mg/ml. ~~Culturing with~~ when absorbance at $OD_{600\ nm}$ became about 0.5, and the shaking culture at 30°C was continued for ~~six~~another 6 hours ~~at 30°C~~. The culture ~~broth~~suspension was centrifuged at 3,000 X g for 20 minutes ~~at 3000 x g~~so as to collect cells and the collected cells~~, which~~ were then suspended in 160 ml of PBS. The suspension was subjected to ~~an ultrasonic generator~~ultrasocination (Ultrasonics Co., Ltd.) so as to ~~disrupt~~crush the cells. ~~The supernatant liquid~~, and the cell lysate was then centrifuged at 7,000 X g for 30 minutes ~~at 7000 x g~~so as to ~~obtain~~collect a soluble protein fraction as a supernatant.

(5) Preparation of OCIF-~~i~~Immobilized ~~a~~Affinity ~~c~~Column

~~Two~~2 g of TSKgel AF-~~Tol~~resyl ~~Toyopal~~TOYOPAL 650 (~~Tosoh Corp~~TOSO CO., LTD.) and 40 ml of 1.0 M potassium phosphate buffer (pH 7.5) containing 35.0 mg of recombinant OCIF, ~~which was~~ prepared ~~according to the~~by a method described in WO 96/26217, were mixed. ~~The mixture was~~ together and gently shaken at 4°C overnight ~~at~~so ~~4°C~~as to ~~effect~~cause a coupling reaction. ~~The reaction mixture was centrifuged to remove the supernatant.~~ To inactivate excessive active ~~residues~~residue, after a supernatant was removed by centrifugation, 40 ml of 0.1 M Tris-HCl buffer (pH 7.5) was added to ~~the~~a precipitated carrier, and the mixture was gently shaken at room temperature for ~~one~~1 hour. ~~The carrier in a column was washed with~~After 0.1 M glycine-HCl buffer ~~(pH 3.3)~~ containing 0.01% Polysorbate 80 and 0.2 M NaCl ~~and~~(pH 3.3) and a 0.1 M sodium citrate buffer ~~(pH 2.0)~~ containing 0.01% Polysorbate 80 and 0.2 M NaCl. ~~The carrier~~ (pH 2.0) were passed through a column (in which the obtained gel was packed) so as to wash it, the column was ~~equilibrated by charging~~washed twice with 10 mM sodium phosphate buffer ~~(pH 7.4)~~ containing 0.01% Polysorbate ~~80.~~80 (pH 7.4) so as to equilibrate it.

(6) Purification of Trx-hOBM ~~using~~by OCIF-~~i~~Immobilized ~~a~~Affinity ~~c~~Column

~~Unless otherwise indicated, purification~~Purification of Trx-hOBM was carried out at 4°C unless otherwise stated. ~~The~~After the above-mentioned OCIF-immobilized affinity carrier (10 ml) and the above-mentioned soluble protein fraction solution (120 ml) ~~prepared~~described in Example 25-(4) were mixed~~. The~~ together, the mixture was gently shaken at 4°C overnight ~~at 4°C~~ in four 50 ml centrifuge~~l~~ tubes ~~using~~by use of a rotor. ~~An Econo-column™~~The carrier in the mixture was filled an EconoColumn (internal diameter: 1.5 cm, length: 15 cm, ~~manufactured by BioRad~~Bio-Rad Co., Ltd.) ~~was filled with the carrier in the mixture~~. ~~The column was charged with~~ 300 ml of PBS containing 0.01% Polysorbate 80, 100 ml of 10 mM ~~sodium~~ phosphate buffer ~~(pH 7.0)~~ containing 0.01% Polysorbate 80 and 2.0 M NaCl (pH 7.0), and 100 ml of 0.1 M glycine-HCl buffer ~~(pH 3.3)~~ containing 0.01% Polysorbate 80 and 0.2 M NaCl (pH 3.3) were passed through the column, in ~~that order~~turn, so as to wash the column. ~~Next~~Then, ~~proteins adsorbed in the column were eluted with~~ 0.1 M sodium citrate buffer ~~(pH 2.0)~~ containing 0.01% Polysorbate 80 and 0.2 M NaCl (pH 2.0) was passed through the column so as to elute proteins adsorbed to the column. ~~The eluate was collected in~~ 5 ml ~~portions~~eluates were fractionated. ~~Each fraction thus collected was immediately neutralized with addition of~~ To the fractions, 10% volume of 2 M Tris-~~buffer~~ solution (pH 8.0) was added so as to immediately neutralize the fractions. ~~Presence~~The presence or absence of Trx-hOBM in the ~~eluted fractions~~each fraction of the eluate was ~~determined according to~~examined in accordance with the method ~~previously~~ described in Example 25-(3) ~~(the binding capability to OCIF)~~. ~~The fractions~~Fractions containing Trx-hOBM were collected and purified further.

(7) Purification of Trx-hOBM by ~~g~~Gel ~~f~~Filtration

About 25 ml of the Trx-hOBM fractions ~~obtained~~described in Example 25-(6) was concentrated using a centrifuge to about 0.5 ml by ~~centrifuging using~~use of a Centriplus R10 and a Centricon R10 (Amicon Co., Ltd.). ~~This~~The concentrated sample was ~~applied~~subjected to a Superose R12 HR 10/30 column (~~1.0~~1.0 ~~×~~X 30 cm, Pharmacia Co., Ltd.) ~~previously~~ equilibrated in advance with PBS containing 0.01% Polysorbate 80. ~~For the separation,~~The column was developed at a flow rate of 0.25 ml/min by using PBS containing 0.01% Polysorbate 80 ~~was used~~ as a mobile phase ~~at a flow rate of 0.25~~

~~ml/min. The eluate~~ so as to fractionate every 0.25 ml of eluates from the column ~~was collected in 0.25 ml portions~~. ~~The~~ Trx-~~hOBM~~OBM in the ~~thus collected~~ fractions was detected by the ~~same~~ method ~~as previously~~ described in ~~the~~ Example 25 (3) and SDS-PAGE. Fractions containing purified Trx-hOBM were collected ~~and~~so as to measure the protein concentration of Trx-~~hOBM was determined~~OBM. The ~~measurement of the~~ protein concentration was ~~carried out~~measured with DC-protein assay kit (Bio-Rad Co., Ltd.) using bovine serum albumin as a reference standard ~~substance using DC Protein assay kit (BioRad Co.).~~

~~<~~[Example 26~~>~~]

~~Osteoclast formation inducing activity of hOBM~~

Osteoclastogenesis Inducing Activity of OBM

phOBM and pcDL-SR-~~α~~α296 were ~~respectively~~ transfected into COS-7 cells ~~using Lipofectamine~~by use of lipofectamine (Gibco Co., Ltd.), respectively. ~~The~~After the cells were cultured ~~for one day~~ in DMEM containing 10% FCS for 1 day, they were trypsinized and seeded in a 24-well plate, ~~plated on~~in which glass cover slips (15 mm round ~~shape, manufactured by~~ Matsunami Co., Ltd.) ~~in 24-well plates~~were seated, at a concentration of 5 X 10^4 cells ~~per~~/well~~,~~ and then cultured for ~~2~~another two days. The culture plate was washed ~~once~~ with PBS. ~~The cells were fixed with~~ once and then PBS containing 1% paraformaldehyde was added, and the cells were incubated at room temperature for 8 minutes so as to fix the cells on the glass cover slips. ~~The~~After the plate ~~on which the~~with fixed cells ~~were attached~~ was washed ~~6 times~~ with PBS~~, then~~ six times, 700 ~~μ~~μl of mouse spleen cells suspended ~~at 1x10^6/ml~~ in ~~α~~α-MEM (containing 10^{-8} M ~~active form~~activated vitamin D_3, 10^{-7} M dexamethasone~~,~~ and 10% fetal bovine serum) in an amount of 1 X 10^6 cells/ml were added to each well. ~~Millicell~~A MILLICELL® PCF (Millipore Co., Ltd.) was set ~~in~~on each well, and a700 ~~suspension~~μl of ST2 cells, suspended in the above ~~mentioned culture~~ medium ~~(~~in a concentration of 4 ~~x~~X 10^4 cells/ml~~)~~ were added~~, 700 μl per well, into~~ to the ~~Millicell~~MILLICELL® PCF ~~followed by incubation~~and cultured at 37°C for 6 days. After ~~the culture~~that, the ~~Millicell~~MILLICELL® PCF was removed~~,~~ and the plate was washed ~~once~~ with PBS~~,~~ ~~and~~once. Then, the cells were fixed ~~with~~for a minute by an acetone-ethanol solution (50:50) ~~for one minute. Then~~, ~~the~~and cells, ~~exhibiting~~having tartaric acid- resistant acid

phosphatase activity (TRAP activity), which is ~~a~~ specific marker ~~for~~of osteoclast, were ~~selectively~~ stained ~~using LEUKOCYTE ACID PHOSPHATASE~~by use of a leukocyte acid phosphatase kit (Sigma Co., Ltd.). ~~As~~Using a ~~result of microscopic observation~~microscope, cells having TRAP-~~positive cells~~activity were not detected in the wells ~~in which~~having COS-7 cells transfected with the pcDL-SR ~~α296 were fixed. In contrast,~~α296, while 65 ~~±~~± 18 (n = 3, average ~~±~~± standard deviation~~, n=3~~) of TRAP positive cells were observed in the wells ~~in which COS-7~~having cells transfected with phOBM ~~were fixed~~. ~~Moreover, expression of~~Further, it was also confirmed that these TRAP positive cells expressed calcitonin ~~receptor was confirmed by~~receptors, since the ~~fact that~~cells showed specific binding to ^{125}I-labeled salmon calcitonin (Amersham Co., Ltd.) ~~specifically bound to these TRAP positive cells~~. ~~Based on~~From these ~~findings~~results, it ~~has been proven~~was revealed that human OBM, ~~which is the~~a protein encoded by the cDNA of the present invention, ~~has~~had an activity to promote osteoclast formation ~~inducing activity~~.

~~<~~[Example 27~~>~~]

~~Osteoclast formation inducing activity~~**Osteoclastogenesis Promoting Activities of Trx-hOBM and ~~s~~Secrete~~dory form~~Type ~~h~~Human OBM**

Mouse spleen cells were suspended in ~~α~~α-MEM containing 10^{-8} M ~~active form~~activated vitamin D_3, 10^{-7} M dexamethasone~~,~~ and 10% fetal bovine serum at a concentration of 2 ~~×~~X 10^6 cells/ml~~. The~~, and 350 µl of the suspension was added to each well of a 24 well plate ~~in the amount of 350 µl per well~~. ~~Each well was then charged with~~After 350 ~~µ~~µl of a solution prepared by diluting purified Trx-~~hOBM~~OBM,(40 ng/ml) with the above ~~mentioned culture~~ medium ~~(40 ng/ml)~~, 350 ~~µ~~µl of a solution prepared by ~~10-fold~~ diluting a conditioned medium ~~which was produced by culturing~~obtained when 293-EBNA cells~~, onto which~~ transduced by pCEPshOBM or pCEP4 were ~~transfected,~~cultured in ~~a culture medium~~ IMDM-10% FCS~~,~~ to be 1/10 with the above-~~mentioned culture~~ medium, or 350 ~~µ~~µl ~~only~~ of the above ~~mentioned culture~~ medium. ~~The Millicell PCF (Mollipore~~ alone was added, a MILLICELL® PCF (Millipore Co., Ltd.) was ~~placed~~set on each well, ~~to which~~and 600 ~~µ~~µl of ST2 ~~cells which were suspended~~cell suspension in the above ~~mentioned culture~~ medium ~~(4 ×~~at a concentration of 4 X 10^4 cells/ml~~)~~ were added to the Millicell® PCF. After the cell were cultured for

~~six~~6 days, the Millicell® PCF was removed~~. The~~, and the plate was washed ~~once~~ with PBS ~~and~~once. Then, after the cells were fixed for 1 minute by an acetone-ethanol solution (50:50) ~~for one minute. Then~~, ~~the~~ cells ~~exhibiting the activity of~~having tartaric acid resistant ~~acidic phophatase~~acid phosphatase activity (TRAP activity) were ~~selectively~~ stained ~~using LEUKOCYTE ACID PHOSPHATASE~~by use of a leukocyte acid phosphatase kit (Sigma Co., Ltd.). ~~The results of microscopic observation revealed that no~~Using a microscope, cells ~~exhibiting the~~having TRAP activity were not detected in the wells ~~to which no~~not containing Trx-hOBM ~~was added~~, ~~whereas~~while 115~~±~~ ± 19 (n = 3, average ~~±~~± standard deviation~~, n=3~~) of TRAP- positive cells were observed in the wells ~~to which~~containing Trx-hOBM ~~was added~~. Similarly, ~~while no~~ cells ~~exhibiting~~having TRAP activity were not detected in the wells ~~to~~containing ~~which~~the conditioned medium of ~~293~~pCEP4 ~~EBNA cells~~ transfected ~~with pCEP4 had been added~~293-EBNA, while 125~~±~~ ± 23 (n = 3, average ~~±~~± standard deviation~~, n=3~~) of TRAP positive cells were observed in the wells ~~to~~containing ~~which~~the conditioned medium of ~~293~~pCEPshOBM ~~EBNA cells~~ transfected ~~with pCEPshOBM had been added~~293-EBNA. ~~Moreover~~Furthermore, ~~expression of calcitonin receptor~~it was also confirmed ~~by the fact~~ that these TRAP positive cells expressed calcitonin receptors, since the cells showed specific binding to ^{125}I- labeled ~~sal~~ermon calcitonin (Amersham Co., Ltd.) ~~specifically binds to~~. From these ~~TRAP positive cells. These~~ results ~~have proven~~, it was revealed that Trx-hOBM and ~~secreted form hOBM exhibit~~secretory-type OBM had an activity to promote osteoclast formation ~~inducing activity~~.

~~<~~[Example 28~~>~~]

Preparation of ~~p~~Polyclonal ~~a~~Antibody

Mouse sOBM or human sOBM, which was used as an ~~immunogen~~immunizing antigen, was ~~prepared according to~~obtained in accordance with the above-mentioned method ~~described above~~. ~~Especially~~That is, mouse sOBM cDNA (cDNA ~~(Sequence ID No. 18) encoding~~which encodes mouse sOBM (~~Sequence~~SEQ ID ~~No.~~NO: 16) ~~which does not have the~~having no membrane binding ~~region of the mouse OBM due to absence of the~~site and lacking amino acids ~~from~~between the N-terminal ~~down to the 72nd~~end and amino acid 72 of mouse OBM; SEQ ID NO: 18) or human ~~sOBM~~OBM cDNA (cDNA ~~(Sequence ID No. 19) encoding~~which encodes human sOBM (~~Sequence~~SEQ ID ~~No.~~NO:

17) ~~which does not have the~~having no membrane binding ~~region of human OBM due to absence of the~~site and lacking amino acids ~~from~~region between the N- terminal ~~down to the~~end and amino acid 71~~st amino acid~~ of human OBM; SEQ ID NO: 19) ~~was ligated~~, together with a Hind III/EcoRV fragment (5.2 kb) of ~~the~~a pSec TagA expression vector ~~pSec TagA~~ (In~~V~~vitrogen Co., Ltd.) ~~including the~~, containing nucleotide sequence ~~encoding~~coding a signal peptide of ~~κ~~κ-chain of immunoglobulin, ~~together with~~and an EcoRI/PmaC~~l~~I fragment (0.32 kb) of OBM cDNA, ~~using~~were subjected to ligation by use of a ligation kit ver. 2 (~~Takara Shuzo Co~~TAKARA SHUZO CO., LTD.). *Escherichia coli* DH5~~-α was~~α were transformed with the reaction product. ~~The plasmids~~Plasmids were purified from the obtained ~~from the resulting~~ ampicillin -resistant ~~strains were purified by the alkali~~clones by alkaline SDS method and ~~digested with an~~cleaved by restriction ~~enzyme~~enzymes so as to select a plasmid ~~with~~having 0.6 ~~Kb~~kb and 0.32 kb of fragments inserted ~~into~~in pSec TagA. ~~The sequence~~As a result of ~~this~~determining the sequences of the plasmid ~~was identified using the Dyedeoxyterminator~~by use of Dye Terminator Cycle Sequencing FS kit (~~product of~~ Perkin Elmer Co.~~)~~, Ltd.~~ As a result~~), it was confirmed that thi~~s~~e plasmid ~~has a sequence~~had sequences encoding mouse or human sOBM. ~~After~~The plasmid was ~~digested~~cleaved ~~with~~by restriction enzymes ~~NheI/XhoI,~~NheI and XhoI and then a fragment (1.0 kb) corresponding to secreti~~on~~ry ~~form~~-type OBM cDNA was ~~recovered~~collected by agarose gel electrophoresis. Thi~~s~~e fragment was inserted into an NheI/XhoI fragment (10.4 kb) of ~~the~~an expression vector pCEP4 (In~~V~~vitrogen Co., Ltd.) ~~using~~by use of a ligation kit~~.~~, and *Escherichia coli* DH5 ~~α was~~α were transformed ~~using~~by use of the reaction product. Plasmids were purified ~~by the alkali SDS~~ from the ~~resulting~~obtained ampicillin -resistant ~~strains. Analyzing these plasmids by digesting with a~~clones by an alkaline SDS method and cleaved by restriction ~~enzyme,~~enzymes and analyzed so as to select a *Escherichia coli* ~~possessing~~clone having a ~~secretion type~~secretory OBM expression plasmid (pCEP sOBM) ~~having~~ with the ~~objective~~ target structure ~~was selected~~. The *Escherichia coli* ~~strain~~clone having the pCEP sOBM was cultured, and the pCEP sOBM was purified ~~using~~by use of a ~~Qiafilter plasmid midy kit~~QIA® Filter Plasmid Midi Kit (~~Qiagen~~QIAGEN Co., Ltd.). Next, 293-EBNA cells ~~were~~was suspended in IMDM containing 10% FCS (IMDM-10% FCS) ~~containing 10% FCS~~ and ~~plated~~seeded ~~onto~~in a ~~24-~~ collagen-coated 24 well plate ~~coated with~~

~~collagen~~ (~~product of~~ Sumitomo Bakelite Co., Ltd.) ~~at a cell density~~in an amount of 2 ~~x~~X 10^5 cells/2 ml/well~~. After~~, and ~~cultur~~inged overnight~~;~~. To the cells ~~were tranformed with~~, 1 ~~μ~~μg of pCEP sOBM or pCEP4 ~~using~~was transfected by use of 4 ~~μ~~μl of ~~L~~lipofectamine (Gibco Co., Ltd.), and ~~further~~the cells were cultured for ~~two~~another 2 days in 0.5 ml of serum-free IMDM or IMDM-10% FCS, thereby collecting a conditioned medium. The ~~culture supernatant was recovered. A cell line~~clones with high ~~productivity~~on of recombinant mouse soluble OBM (msOBM) or human soluble OBM (hsOBM) ~~was~~were screened ~~as follows~~in the following manner. ~~Sodium bicarbonate~~After sodium hydrogen carbonate was added to the ~~culture supernatant which is assumed~~conditioned medium seemed to contain msOBM or hsOBM ~~to~~at a final concentration of 0.1 M~~. One, hundred~~100 ~~μ~~μl of the ~~culture supernatant~~conditioned medium was added to each well ~~in~~of 96- well immunoplates (Nunc Co.~~) and allowed to stand~~, Ltd.) and the plate was left to stand at 4°C overnight ~~at 4°C, thereby~~so as to solid-phase the msOBM or hsOBM in the ~~culture supernatant was immobilized~~conditioned medium on each well. ~~To each well~~Then, 200 ~~μ~~μl of ~~Block Ace™~~BLOCKACE (Snow Brand Milk Products Co., Ltd.) solution diluted ~~four-fold~~to 4 times with PBS (B-PBS) was added ~~and the plates were allowed to stand for two hours~~to each well of the plate and the plate was left to stand at room temperature for 2 hours. After washing ~~each well in the plates~~ three times with PBS ~~(P-PBS)~~ containing 0.1% Polysorbate ~~20,~~20 (P-PBS), 100 ~~μ~~μl of ~~each~~ recombinant OCIF (rOCIF) solution ~~(~~3diluted stepwise (0-100 ng/ml) ~~diluted serially~~ with ~~P~~B-PBS was added to each well ~~in~~and the ~~plates. The plates were allowed~~plate was left to stand at 37°C for ~~two~~2 hours ~~at 37°C~~. After washing ~~the plates~~ three times with PBS, 100 ~~μ~~μl of ~~a~~ peroxidase- labeled anti-OCIF polyclonal antibody (WO 96/26217), diluted with B-PBS, was added to each well~~. After allowing~~ and the plate was left to stand at 37°C for ~~two~~2 hours ~~at 37°C, the wells were washed~~. After washing six times with P-PBS~~. Then~~, 100 ~~μ~~μl of TMB solution (TMB Soluble ~~Reagent~~Agent, High ~~S~~sensitivity, Scy~~T~~tek Co., Ltd.) was added to each well ~~in the plates~~ and ~~the plates were allowed~~left to stand at room temperature for about 10 minutes~~, subsequently the reaction was terminated by adding~~. Thereafter, 100 ~~μ~~μl of ~~a stopping solution (~~Stopping Reagent~~, ScyTek Co.) to each~~ (Scytek Co., Ltd.) was added to each well. The absorbance of each well~~. Absorbance~~ at 450 nm ~~of each well~~ was measured

~~using~~by means of a microplate reader. ~~It was confirmed that~~In the plate having the solid-phased protein derived from conditioned medium of the clone producing msOBM or hsOBM, the absorbance significantly increased ~~remarkably~~ in proportion to the concentration of the ~~added~~ OCIF. As ~~in~~for the ~~plates in which msOBM or hsOBM in the culture supernatant of the cell line~~clones producing msOBM or hsOBM ~~was immobilized therein. The cell line that exhibited~~, clones indicating a high rate of increase in the absorbance ~~was~~were selected as ~~a a strain with high productivity~~highly producing clones thereof. ~~Thus related 293 EBNA cells with high productivity~~Each of the highly producing clones of msOBM or hsOBM selected in the above mentioned manner were mass-cultured ~~on a large scale in an~~by use of IMDM ~~medium~~ containing 5% FCS~~, using~~ as a medium in 25 T-flasks (T-225). After the cells ~~reached~~grew to confluency, ~~a~~100 ml of fresh ~~culture~~ medium was added to each T-~~225 flask in the amount of 100 ml per~~255 flask and the cells were further cultured for 3- or 4 days, ~~to collect the culture supernatant~~and then a conditioned medium was collected. ~~These procedures were repeated four~~By repeating this procedure 4 times ~~to obtain~~, 10 ~~L~~liters of the ~~culture~~conditioned ~~supernatant~~medium containing msOBM ~~or~~and 10 liters of the conditioned medium containing hsOBM were obtained. ~~Purified msOBM (~~About 10 mg~~) or hsOBM (~~ of purified msOBM and about 12 mg~~)~~ of purified hsOBM, which ~~shows homogeneous band~~were uniform (molecular weight: 32 kDa) ~~on~~in terms of SDS-polyacrylamide ~~gel~~ electrophoresis, were obtained ~~from~~by carrying out purification on the ~~culture supernatant by means of~~above-obtained conditioned medium with affinity chromatography ~~on~~using an ~~OCIF~~rOCIF-immobilized column and gel filtration chromatography ~~according to~~in accordance with the method described in ~~e~~Examples 25-(6) and ~~25~~(7). ~~Each~~The ~~thus~~-obtained purified ~~preparations~~samples ~~was~~were used as ~~an antigen for immunization~~immunizing antigens. ~~Each protein antigen~~The obtained ~~was~~antigens each were dissolved in phosphate buffered saline (PBS) ~~to~~at a concentration of 200 ~~μ~~μg/ml and ~~emulsified~~then the solution was mixed with an equ~~i~~valent ~~volume~~amount of Freund's complete adjuvant so as to be emulsified. ~~One~~1 ml of ~~the~~each emulsion was subcutaneously ~~immunized~~administered to ~~each of~~ three Japanese white rabbits at intervals of about ~~once every~~one week so as to immunize the rabbits. ~~A booster injection~~An antibody titer was ~~given~~measured, and when the antibody titer

reached a ~~peak~~maximum, a booster was carried out. ~~Whole~~10 days after the booster, all blood was collected ~~10 days thereafter~~from all the rabbits. ~~The serum~~Antiserum was diluted to two~~-fold~~ times with~~-a~~ binding buffer for ~~p~~Protein A ~~sepharose~~Sepharose® chromatography (~~BioRad~~Bio-Rad Co., Ltd.) and ~~applied~~then added to a ~~p~~Protein A column equilibrated with the ~~same~~above buffer. After ~~washing~~ the column ~~extensively~~was efficiently washed with the ~~same~~above buffer, ~~the~~an anti-sOBM antibody adsorbed to the column was eluted ~~with~~by an elution buffer (~~BioRad~~Bio-Rad Co., Ltd.) or 0.1 M glycine-HCl buffer~~,~~ (pH ~~3.0~~2.9 to 3.0). ~~To~~In order to immediately neutralize the antibody-containing eluate ~~immediately~~, the ~~eluate~~eluted solution was fractionated ~~using~~by use of a test tubes containing a small amount of 1.0 M Tris-HCl (pH 8.0). The antibody eluate was dialyzed ~~against~~in PBS at 4°C overnight ~~at 4°C~~. The ~~antibody content~~amount of protein in the antibody solution was measured ~~by~~in accordance with the Lowry method using bovine IgG as a standard ~~protein~~. Thus, ~~about 10 mg of~~the purified immunoglobulin (IgG) containing the polyclonal antibody of the present invention was obtained in an amount of about 10 mg per 1 ml of rabbit antiserum ~~was obtained~~.

~~<~~[Example 29~~>~~]

Measurements of OBM and sOBM by ELISA Using Polyclonal Antibody

Sandwich ELISAs, using the rabbit anti-hsOBM polyclonal antibody~~A sandwich ELISA was constructed using the rabbit anti-human sOBM polyclonal antibody~~ obtained in Example 28 as ~~the~~ a solid phase antibody and ~~enzyme~~ as an enzyme labeled antibody, were constructed. ~~Peroxidase~~As enzyme labeling, peroxidase (POD) ~~labeled antibody~~labeling was ~~prepared according to the~~carried out in accordance with a method of Ishikawa *et al.* (Ishikawa *et al.*~~,~~: J. Immunoassay, Vol. 4, 209~~-327, 1983~~ to 327, 1983). The anti-~~human sOBM~~hsOBM polyclonal antibody obtained in~~-the~~ Example 28 was dissolved in a 0.1 M $NaHCO_3$ ~~to~~ solution at a concentration of 2 ~~μg/ml. One hundred~~μg/ml, and 100 ~~μ~~μl of the resulting solution was added to each well ~~in~~of 96-well immunoplates (Nunc Co.~~), which was then allowed~~, Ltd.) and the plate was left to stand at room temperature overnight. ~~Next~~Then, 200 ~~μ1~~μl of 50% ~~Block Ace™~~BLOCKACE (Snow Brand Milk Products Co., Ltd.) was added to each well, and the plate~~s were allowed~~ was left to stand ~~for one hour at~~ at room temperature~~.~~ for 1 hour. ~~The~~ Each wells ~~were~~ was washed ~~three times~~ with PBS containing 0.1% ~~P~~polysorbate 20 (washing

buffer).Human three times. The purified human OBM, which was expressed according toin the method ofsame manner as in Example 26 and was purified according toin the method ofsame manner as in Example 2. The purified human OBM2, and the purified human sOBM, preparedobtained in example 28 were seriallyExample 28, was diluted stepwise with the firstprimary reaction buffer (0.2 M Tris-HCl buffer, pH 7.2, containing 40% Block AceBLOCKACE and 0.1% Polysorbate 20polysorbate 20, pH 7.2), respectively, and 100 µl of the diluted solution wasµl of each diluent were added to each well in. After the plates. The plates were allowedplate was left to stand at room temperature for two2 hours, andeach well was washed three times with the above-mentioned washing buffer three times. Subsequently, 100 µlµl of POD- labeled anti-human sOBM polyclonal antibody, diluted 1000-fold1,000 times with the secondsecondary reaction buffer (0.1 M Tris-HCl buffer, pH 7.2, containing 25% Block AceBLOCKACE and 0.1% Polysorbate 20polysorbate 20, pH 7.2) was added to each well inand the plates. After the plates were allowedplate was left to stand at room temperature for two2 hours,. eEach well was washed three times with the washing buffer three times. Next, 100 µlµl of enzyme substrate solution (TMB, ScyTek Co., Ltd.) was added to each well in the plates, and the plates were allowed was left to stand at room temperature for 10 minutes, followed by the addition of . 100 µlµl of a reaction terminationstopping solution (Stopping reagent, ScyTek Co., Ltd.) was added to each well so as to stop the enzyme reaction. The absorbance at 450m nm of each well was measured usingby use of a microplate reader. The results are shown in FigureFig. 26. The sandwich ELISA, using athe rabbit anti-human sOBM polyclonal antibody recognized, almost equally detected both human sOBM (molecular weight;: about 32 kDa) and human OBM (molecular weight;: about 40 kDa), with aand measurement sensitivity ofwas about 12.5 *X 10^{-3} pmol/ml (human OBM: about 500 pg/ml; for human sOBM:OBM, about 400 pg/ml for human sOBM). The measurementIt was revealed that measurements of mouse sOBM and mouse OBM by ELISA using the rabbit anti-mouse sOBM polyclonal antibody obtained in the Example 28 was able tocould be carried outmade in the same manner. It was confirmed that an extremely as described above, measurement sensitivity in measuring mouse OBM or mouse sOBM was similar with that in human OBM or human sOBM, and a very small amount of mouse sOBM or mouse

OBM ~~can be measured with almost the same sensitivity as described above.~~could be measured.

~~As mentioned above, the~~
As described above, since the present anti-human sOBM ~~polyclonal antibody of the present invention prepared in the Example 28 can equally recognize both the~~ polyclonal antibody obtained in Example 28 recognized both human sOBM and human OBM ~~antigens. Therefore, the antibody was~~ as antigen equally, it was named an anti-~~human OBM/~~human OBM/sOBM polyclonal antibody. Meanwhile, since the anti-mouse sOBM polyclonal ~~antibody. Similarly, the anti-mouse~~ antibody obtained in Example 28 recognized both mouse sOBM and mouse OBM as antigen equally, it was named an anti-mouse OBM/sOBM polyclonal ~~antibody prepared in the Example 28 can equally recognize both the mouse sOBM and mouse OBM antigens. This antibody was therefore named anti-mouse OBM/sOBM polyclonal antibody.~~

~~<Example 30>~~

~~Preparation of monoclonal antibody~~ antibody.

[Example 30]

Preparation of Monoclonal Antibody

The purified human sOBM ~~prepared~~ obtained in ~~the~~ Example 28 was used as ~~the antigen for immunization~~ an immunizing antigen. The purified human sOBM was dissolved in ~~physiological~~phosphate buffered saline ~~solution to~~at a concentration of 10 ~~μg/ml and emulsified by mixing with an equivalent volume of Freund's complete adjuvant~~μg/ml. To the prepared human sOBM solution, an equal amount of Freund's complete adjuvant was added so as to emulsify it. ~~The emulsion was intraperitoneally~~Thereafter, 200 μl of the antigen was administered ~~to BALB~~into the abdominal cavity of each Balb/c ~~mice~~mouse at an interval of one week for a ~~dose~~total of ~~200 μl~~ three times~~, once a week,~~ so as to immunize the mice. ~~Next~~Then, ~~the equivalent volume of the Freund's complete adjuvant was added~~ to a physiological saline solution containing 5 ~~μ~~μg/ml of the human sOBM, an equal amount of Freund's incomplete adjuvant was added so as to fully emulsify it, and 200 μl of the ~~mixture was sufficiently emulsified. This~~ emulsion was ~~injected intraperitoneally to BALB~~administered to each

of the above Balb/c mice at ~~a dose of 200 μl, once a~~one week intervals for a total of four ~~weeks for immunization~~times so as to further immunize the mice. ~~One~~After the passage of one week ~~after~~from the fourth additional immunization, 100 ~~μl~~μl of ~~a~~phosphate ~~physiological~~buffered saline solution containing 10 ~~μ~~μg/ml of the human sOBM was ~~intravenously~~parenterally administered to each of the ~~BALB~~Balb/c mice ~~as a~~for booster. ~~After three days~~On the 3rd day after the final immunization, the spleen was ~~extracted~~removed, and spleen cells were separated~~. The spleen cells were~~ and fused with mouse myeloma cells~~,~~ P3x63-~~Ag~~AG8.653 ~~according to~~in accordance with a ~~conventional~~known method (Koehler, G. and Milstein, C., Nature, 256, 495 (1975)). ~~The suspended fused cells were~~After completion of the fusion, the cell suspension was cultured ~~for 10 days~~ in ~~an~~a HAT medium containing hypoxanthine, aminopterin~~,~~ and thymidine for 10 days. After the myeloma cells ~~were dead~~perished and hybridomas appeared, the ~~HAT~~ medium was replaced with an HT medium obtained by removing aminopterin ~~free~~ from the HAT medium, and the ~~cell~~ culture was continued.

~~<~~[Example 31~~>~~]

Selection ~~of~~and Cloning of Hybridoma

Since the appearance of the hybridoma ~~and cloning~~Appearance of hybridomas was ~~recognized 10 days after~~seen on the 10th day from the start of the cell fusion and culturing in Example ~~30. Monoclonal antibodies~~30, a high affinity antibody recognizing ~~the~~ human sOBM ~~with high affinity and hybridomas~~and hybridoma producing ~~these~~ antibod~~ies~~y were selected ~~according to~~in the means of the following ~~procedure using the~~ improved solid phase ELISA ~~which is described below~~. ~~In addition~~Further, to select ~~the~~an anti-OBM monoclonal antibody ~~which recognizes~~recognizing both of human sOBM and mouse sOBM, the mouse sOBM ~~prepared~~obtained in ~~the~~ Example 27 ~~was used in addition to~~ as well as human sOBM was used as ~~the~~an antigen ~~for~~in the solid phase ELISA. ~~The human~~Human sOBM and mouse sOBM ~~were~~each ~~respectively~~was dissolved in ~~a~~ 0.1 M sodium ~~bicarbonate~~hydrogen carbonate solution at a concentration of 5 ~~μ~~μg/ml~~. Fifty ml~~, and 50 μl of each antigen solution was added to each well ~~in~~of a 96-~~-~~well immunoplates (Nunc Co.~~)~~, Ltd.~~ The plates were allowed~~), and the plate was left to stand at 4°C overnight so as to ~~immobilize~~attach the antigens. The antigen solution in each well was discarded~~. Each well was then filled with~~, and 200 ~~μl~~μl of 50% ~~Block~~

~~Ace™~~BLOCKACE (Snow Brand Milk Products Co., Ltd.) ~~and allowed~~was added to each well. The plate was left to stand at room temperature for ~~one~~1 hour so as to cause blocking. After each well was washed with a phosphate buffered saline ~~solution (PBS-P)~~ containing 0.1% ~~P~~polysorbate 20, 40 ~~μ1~~μl of ~~calf~~bovine serum (~~Hiclone Inc~~Hyclone Co., Ltd.) was added to each well. ~~Subsequently~~Then, 10 ~~μ1~~μl of ~~each~~ hybridoma ~~culture supernatant~~conditioned medium was added to each well and ~~each~~the ~~well~~plate was ~~incubated~~left to stand under a serum concentration of 80% at room temperature for ~~two~~2 hours ~~in the presence of 80% calf serum~~so as to cause reaction. ~~The~~An object of the solid phase ELISA in the presence of 80% ~~calf~~ serum is to select ~~a hybridoma which produce~~ an antibody ~~which can detect~~capable of binding to a ~~very~~ small amount of human sOBM or mouse sOBM even in ~~a solution containing high concentration~~the presence of protein and ~~in the presence of an immunoreaction interfering~~a serum-derived immune reaction inhibiting substance ~~derived from serum~~in high concentration, ~~i.e.~~that is, to select a hybridoma ~~which can produce~~producing an antibody ~~with a~~having high affinity for human sOBM or mouse sOBM. After completion of the reaction at room temperature for ~~two~~2 hours, the plates ~~were~~was washed with PBS--P, and ~~subsequently,~~ 50 ~~μ1~~μl of diluent of peroxidase- labeled anti-mouse IgG (KPL ~~Co~~CO., LTD.) diluted ~~5000-fold~~to 5,000 times with a physiological saline solution containing 25% ~~Block Ace~~BLOCKACE was added to each well. ~~After~~, and the ~~reaction~~plate was left to stand at room temperature for ~~two~~2 hours~~,~~ so as to cause a reaction. After the plate was washed with PBS-P three times ~~with PBS-P. After the addition of~~, 50 ~~μ1~~μl of ~~an enzyme~~ substrate solution (TMB, ScyTek Co., Ltd.) was added to each well~~, the reaction was continued~~ and left to stand at room temperature for ~~five~~5 minutes. ~~The enzymatic reaction was stopped by the addition of~~Then, 50 ~~μ1~~μl of a ~~termination solution (~~reaction stopping reagent (Stopping Reagent, ScyTek Co., Ltd.) was added so as to terminate the enzyme reaction. ~~Hybridomas which produce antibodies recognizing human sOBM or mouse sOBM were selected by measuring~~The absorbance at 450 nm of each well ~~using~~was measured by use of a microplate reader (~~Immune Reader~~IMMUNOREADER NJ~~2000™~~,2000, Nippon Inter~~M~~med Co.~~)~~, Ltd.~~ Hybridomas~~) so as to select a hybridoma producing ~~antibodies exhibiting~~an antibody which recognizes human sOBM or mouse sOBM. The hybridomas showing particularly high absorbance (OD_{450nm}) were selected.

~~Cloning of these hybridomas~~ and repeatedly cloned 3 to 5 times by a limiting dilution method ~~was repeated 3~~so as to ~~5 times to establish stable~~established hybridomas producing antibody stably. ~~Hybridomas exhibiting particularly high~~Out of the obtained hybridomas, hybridomas having higher antibody productivity were selected ~~among the established antibody producing hybridoma clones~~.

<[Example 32>]

Production and ~~p~~Purification of ~~m~~Monoclonal ~~a~~Antibody

The ~~antibody producing hybridomas~~antibodies obtained in ~~the~~ Example 31, ~~i.e. high affinity antibody producing~~ that is, the hybridoma producing an antibody which recognizes human sOBM with high affinity and the hybridoma producing the antibody which ~~produces an antibody showing~~has a cross-reactivity ~~to the~~with mouse sOBM were cultured, ~~respectively.~~ and ~~E~~each hybridoma was implanted ~~intraperitoneally to BALB~~in the abdominal cavity of a Balb/c ~~mice~~-based mouse which had been given pristane (Aldrich Chemical Co., Ltd.) about a week before, in an amount of 1 ~~×~~X 10^6 cells ~~per~~ /mouse~~) to which pristan (Aldorich Co.) was administered one week previously~~. After about 2- or 3 weeks, accumulated ascites ~~were collected. The~~was sampled so as to obtain ascites containing the monoclonal ~~antibody, which recognizes~~antibody recognized human sOBM ~~of the present invention~~ or ~~both~~ the monoclonal antibody recognizing human sOBM and mouse sOBM. Purified monoclonal antibodies were obtained from the ascites using Protein A column (Pharmacia Co., Ltd.) chromatography in ~~the ascites, was purified according to the purification~~accordance with the method ~~of~~for purifying an anti-OBM/sOBM polyclonal ~~antibodies using a Protein A column~~antibody described in ~~the~~ Example 28. ~~The purified monoclonal antibody was thus obtained from the ascites by Protein A column chromatography (Pharmacia Co.).~~

<[Example 33>]

~~Antigen specificity of monoclonal antibody~~

Antigenic Specificity of the Monoclonal Antibody

The antigenic specificity~~ies~~ of ~~a~~-monoclonal ~~antibody~~antibodies, which specifically recognize~~s~~d human sOBM, and ~~the~~of monoclonal ~~antibody~~antibodies, ~~exhibiting~~having cross-reactivity ~~to both the~~with human sOBM and mouse sOBM ~~was investigated~~, were examined using human sOBM, ~~human~~-intact human OBM having a

membrane binding ~~region~~site, mouse sOBM, and ~~mouse~~ intact ~~sOBM~~mouse OBM having a membrane binding ~~region~~site as antigens. ~~More than thirty kinds~~Although over 30 types of monoclonal antibod~~y~~ies were obtained~~.~~, ~~T~~the results ~~on several~~of representative monoclonal antibodies are shown in Table 1. As a result, it was ~~found~~revealed that most of anti-human sOBM monoclonal antibodies which specifically recognized human sOBM ~~also recognize the human~~recognized even intact human OBM having a membrane binding ~~region, but~~site and did not ~~the~~recognize mouse ~~OBM~~sOBM and ~~the mouse~~ intact mouse OBM ~~which has~~having a membrane binding ~~region~~site. ~~On the other hand~~

Meanwhile, ~~it was found that only~~ a few monoclonal antibodies recognizing both ~~the~~of human sOBM and mouse sOBM were also obtained and it was found that these antibodies ~~exhibit~~had cross-reactivity ~~to both the~~with human OBM and mouse OBM. These results ~~show~~indicate that ~~there is a common antigen recognizing sites, namely a common epitopes, in both the~~ human OBM and mouse OBM had a common antigen recognition site, i.e., epitope. ~~Based on the fact that the~~Since an anti-human sOBM monoclonal antibody prepared ~~using the~~by use of human sOBM as an ~~immune~~ antigen also equally recognize~~s~~d human OBM ~~having~~, which was a membrane binding ~~region. Anti-human sOBM~~intact protein, the monoclonal antibody was named ~~the~~an anti-human OBM/sOBM monoclonal antibody.

~~TABLE 1~~

~~Antibody~~	~~Antigen~~			
	~~hsOBM~~	~~hoBM~~	~~MsOBM~~	~~mOBM~~
~~H-OBM-1~~	~~+~~	~~+~~	~~-~~	~~-~~
~~H-OBM-2~~	~~+~~	~~+~~	~~-~~	~~-~~
~~H-OBM-3~~	~~+~~	~~+~~	~~-~~	~~-~~
~~H-OBM-4~~	~~+~~	~~+~~	~~-~~	~~-~~
~~H-OBM-5~~	~~+~~	~~+~~	~~-~~	~~-~~
~~H-OBM-6~~	~~+~~	~~+~~	~~-~~	~~-~~
~~H-OBM-7~~	~~+~~	~~+~~	~~-~~	~~-~~
~~H-OBM-8~~	~~+~~	~~+~~	~~-~~	~~-~~
~~H-OBM-9~~	~~+~~	~~+~~	~~+~~	~~+~~
~~H-OBM-10~~	~~+~~	~~+~~	~~-~~	~~-~~
~~H-OBM-11~~	~~+~~	~~+~~	~~-~~	~~-~~
~~H-OBM-12~~	~~+~~	~~+~~	~~-~~	~~-~~
~~H-OBM-13~~	~~+~~	~~+~~	~~+~~	~~+~~
~~H-OBM-14~~	~~+~~	~~+~~	~~-~~	~~-~~

Table 1

Antibody	Antigen hsOBM	hOBM	msOBM	mOBM
H-OBM 1	±	±	-	-
H-OBM 2	±	±	-	-
H-OBM 3	±	±	-	-
H-OBM 4	±	±	-	-
H-OBM 5	±	±	-	-
H-OBM 6	±	±	-	-
H-OBM 7	±	±	-	-
H-OBM 8	±	±	-	-
H-OBM 9	±	±	±	±
H-OBM 10	±	±	-	-
H-OBM 11	±	±	-	-
H-OBM 12	±	±	-	-
H-OBM 13	±	±	±	±
H-OBM 14	±	±	-	-

(hsOBM: human soluble OBM, hOBM: human membrane ~~bonding type~~binding OBM, msOBM: mouse soluble OBM, mOBM: ~~human~~mouse membrane ~~bonding type~~binding OBM)

<[Example 34>]

~~Determination of~~

Tests of eClass and sSubclass ~~of~~ of mMonoclonal aAntibody

The class and subclass of the monoclonal antibody of the present invention were determined by ~~the immunoglobulin class and subclass analysis kit~~use of the Immunoglobulin Class/Subclass Analytical Kit (Amersham Co., Ltd.) ~~according to the~~. The tests were conducted in accordance with a protocol ~~indicated~~provided in the kit. The results ~~on~~of representative monoclonal antibodies are shown in Table 2. ~~As shown in Table 2, the~~The majority of anti-human OBM/sOBM monoclonal antibodies ~~were~~had IgG_1, ~~the others were~~and some antibodies having IgG_{2a} ~~and~~or IgG_{2b} were also found. ~~Light chains for~~Further, all of the antibodies ~~were κ chains~~had κ chain as a light chain.

~~TABLE 2~~

~~Antibody~~	~~IgG_1~~	~~IgG_{2a}~~	~~IgG_{2b}~~	~~IgG_3~~	~~IgA~~	~~κ~~
~~H-OBM 8~~	-	+	-	-	-	+
~~H-OBM 9~~	+	-	-	-	-	+
~~H-OBM 10~~	+	-	-	-	-	+
~~H-OBM 11~~	+	-	-	-	-	+
~~H-OBM 12~~	-	-	+	-	-	+

H-OBM 13	+	-	-	-	-	+
H-OBM 14	+	-	-	-	-	+

Table 2

<

Antibody	IgG_1	IgG_{2a}	IgG_{2b}	IgG_3	IgA	κ
H-OBM 8	-	+	-	-	-	+
H-OBM 9	+	-	-	-	-	+
H-OBM 10	+	-	-	-	-	+
H-OBM 11	+	-	-	-	-	+
H-OBM 12	-	-	+	-	-	+
H-OBM 13	+	-	-	-	-	+
H-OBM 14	+	-	-	-	-	+

[Example 35>]

Measurement of ~~the dissociation constant (K_d~~Dissociation Constant (K_D value) ~~of monoclonal antibody~~for the Monoclonal Antibodies

The dissociation ~~constant of the~~constants for monoclonal antibod~~y~~ies ~~was~~were measured ~~according to~~in accordance with a known method (Betrand Friguet *et al.*: Journal of Immun~~un~~ological Methods, 77, 305- to 319, 1986). That is, the purified antibody obtained in ~~the~~ Example 32 was diluted at 5 ng/ml with 0.4 M Tris-HCl ~~buffer (a primary buffer, pH 7.4)~~ containing 40% ~~Block Ace~~BLOCKACE and 0.1% ~~P~~polysorbate 20 ~~to give a concentration of 5 ng/ml. The solution was mixed with~~(pH 7.4, primary buffer) and an equi~~val~~ent ~~volume~~amount of ~~a diluted solution~~diluent of the purified human soluble OBM (hsOBM) obtained in Example ~~28 in~~28, prepared with the primary buffer ~~with a~~ at stepwise-concentration ~~range of~~from 6.25 ng/ml to 10 ~~μ~~μg/ml. ~~The mixture was allowed~~, was added and the solution was left to stand at 4°C for 15 hours ~~at~~so ~~4°C~~as to bind the ~~hsOBM to the~~ monoclonal antibody to hsOBM. After 15 hours, ~~the~~an antibody ~~not bound~~unbound to ~~the hsOBM (10 μg/ml, 100 μl/well)~~ hsOBM was measured ~~using an immobilized~~by solid phase ELISA with solid-phased hsOBM (10 μg/ml, 100 μl/well) so as to calculate the dissociation constant of the monoclonal antibody to ~~the~~ hsOBM. ~~In addition,~~Further, the affinity ~~to~~for msOBM of ~~an antibody, which is a~~ monoclonal ~~antibody for the hsOBM and also exhibits the~~antibodies, having cross-reactivity ~~to~~with mouse soluble OBM (msOBM) and hsOBM, was also measured ~~according to the same method except for~~by using msOBM ~~instead of~~in place of hsOBM

at the above-mentioned method. Particularly, the ~~hsOBM. Dissociation constant~~results of particular antibodies, which ~~exhibit~~had high affinity ~~to~~for each ~~antigen~~of the antigens and ~~are~~were useful ~~for~~in enzymatic immunoassay ~~and~~, binding assay and such, are shown in Table 3.

Table 3

~~Antibody~~	~~Subclass~~	~~Antigen~~	~~Dissociation constant Kd (M)~~
~~H-OBM 1~~	~~IgG_1 (κ)~~	~~hsOBM~~	~~$1 X 10^{-11} < kd < 1 x 10^{-10}$~~
~~H-OBM 4~~	~~IgG_1 (κ)~~	~~hsOBM~~	~~$1 x 10^{-11} < kd < 1 x 10^{-10}$~~
~~H-OBM 9~~	~~IgG_1 (κ)~~	~~hsOBM~~	~~$1 x 10^{-9} < kd < 1 x 10^{-8}$~~
~~H-OBM 9~~	~~IgG_1 (κ)~~	~~msOBM~~	~~$1 x 10^{-8} < kd < 1 x 10^{-7}$~~

Antibody	Subclass	Antigen	Dissociation Constant Kd (M)
H-OBM 1	IgG_1 (κ)	hsOBM	$1 X 10^{-11} < Kd < 1 X 10^{-10}$
H-OBM 4	IgG_1 (κ)	hsOBM	$1 X 10^{-11} < Kd < 1 X 10^{-10}$
H-OBM 9	IgG_1 (κ)	hsOBM	$1 X 10^{-9} < Kd < 1 X 10^{-8}$
H-OBM 9	IgG_1 (κ)	msOBM	$1 X 10^{-8} < Kd < 1 X 10^{-7}$

As a result, ~~the dissociation constants (Kd) of~~it was found that H-OBM 1 and H-OBM 4 which ~~are the~~were specific antibodies ~~specific to~~ for human soluble OBM (hsOBM) ~~were in~~showed a dissociation constant on the order of 10^{-11} M, indicating ~~the~~that they had very high affinity ~~to~~for hsOBM. ~~On the other hand~~Meanwhile, the Kd value of ~~the antibody~~ H-OBM 9 which ~~recognizes~~was an antibody recognizing both ~~the~~ hsOBM and mouse soluble OBM (msOBM) was ~~in~~on the order of 10^{-8} M with respect to msOBM and ~~in~~on the order of 10^{-9} M with respect to hsOBM. ~~In addition~~Further, ~~the dissociation constant of the other~~regarding H-OBM 13, which was another antibody ~~which recognizes~~recognizing both antigens shown in ~~the~~ Table 1, ~~i. e.~~ the dissociation constants of H-OBM 13 ~~for each antigen, was the same as that~~with respect to both antigens were almost identical with those of H-OBM 9, and ~~these~~since ~~two~~both antibodies ~~belong to~~had the same subclass~~. These findings suggest the~~, a possibility was suggested that they ~~are~~were the ~~identical antibodies which recognize~~same antibody recognizing the same epitope ~~of each antigen~~.

~~<~~[Example 36~~>~~]

Method for Measuring ~~method of human~~Human OBM and sOBM by ~~s~~Sandwich ELISA ~~u~~Using ~~a~~Anti-~~h~~Human OBM/sOBM ~~m~~Monoclonal ~~a~~Antibod~~ies~~y

~~A sandwich~~Sandwich ELISA was constructed ~~using~~by use of the two types of high affinity monoclonal antibodies obtained in Example 35, i.e., H-OBM 1 and H-OBM 4, ~~respectively~~ as a solid phase antibody and an enzyme- labeled antibody, respectively. ~~Labeling of~~Maleimide Activated Peroxidase Kit (Pierce Co., Ltd.) was used for labeling the antibody ~~was carried out using a maleimide activated peroxidase kit (Piers Co.)~~. ~~The~~ H-OBM 1 antibody~~, H-OBM 1,~~ was dissolved in ~~a~~ 0.1 M sodium ~~bicarbonate~~hydrogen carbonate solution ~~to~~at a concentration of 10 ~~μ~~μg/ml, and 100 ~~μ~~μl of the resulting solution was added to each well ~~in~~of 96-well immunoplates (Nunc ~~company~~Co., Ltd.). ~~After being allowed~~The plate was left to stand at 4°C overnight ~~at~~so ~~4°C~~as to ~~immobilize~~attach the antibody~~,~~. After the solution in each well was discarded ~~and~~, 300 ~~μ~~μl of 50% ~~Block Ace™ solution~~BLOCKACE was added to each well, ~~in~~and the ~~plates. Each well in the plates~~plate was ~~blocked by allowing~~left to stand at room temperature for ~~two~~2 hours so as to cause blocking. After the blocking, the plates ~~were~~was washed with phosphate buffered saline containing 0.1% ~~P~~polysorbate 20 (PBS-P). Human ~~OBM (hOBM)~~ soluble sOBM and human ~~soluble~~ OBM ~~(hsOBM)~~each were ~~respectively diluted with~~dissolved in 0.4 M Tris-HCl ~~buffer,~~ (pH ~~7.4,~~7.4) containing 40% ~~Block Ace™~~BLOCKACE (Snow Brand Milk Products Co., Ltd.) and 0.1% ~~P~~polysorbate 20 (Wako Pure Chemicals ~~Co.~~Industries, Ltd.) (~~the first~~primary reaction buffer) and diluted so as to prepare test samples with various concentrations. ~~These test samples with different~~100 μl of each of test sample, prepared at various concentrations, ~~were~~was added to each well ~~in the amount of 100 μl per well~~, and ~~reacted~~the plate was left to ~~the antibody, H-OBM 1 immobilized on each well by incubating~~stand at room temperature for ~~two~~2 hours~~.~~ so as to cause a reaction. ~~After two hours, the plates were~~Thereafter, the plate was washed with PBS-P~~. Next,~~, and 100 ~~μ~~μl ~~of a solution~~ of POD- labeled H-OBM 4 antibody ~~in~~diluted with 0.2 M Tris-~~HCl buffer,~~ HC (pH ~~7.4,~~ 7.4) containing 25% ~~Block Ace™~~BLOCKACE and 0.1% ~~P~~polysorbate 20 (~~the second~~secondary reaction buffer) was added to each well~~, followed by further incubating~~. The plate was left to stand at room temperature for ~~two~~2 hours so as to cause a reaction. ~~The plates were then~~After the plate was washed with PBS-P ~~and~~, 100 ~~μ~~μl of ~~an enzyme~~ substrate solution (TMB, ScyTek

Co., Ltd.) was added to each well ~~to start enzyme reaction. The enzyme reaction was terminated by~~so as to develop color in the ~~addition~~wells, ~~of~~and 100 ~~μ~~μl of ~~a~~ reaction ~~termination~~stopping solution (stopping reagent, ScyTek Co., Ltd.) was added to each well so as to stop the enzyme reaction. The absorbance ~~of each well~~ at 450 nm of each well was measured ~~using~~by use of a microplate reader. The results are shown in ~~Figure~~ Fig. 27.

As a result, it was ~~confirmed~~revealed that the sandwich ELISA constructed ~~using~~by use of the two types of high affinity anti-human OBM/sOBM monoclonal antibodies obtained in Example 35, i.e., H-OBM 1 and H-OBM ~~4 with high affinity for~~4, detected human OBM/ and human sOBM ~~prepared in the Example 35, equally recognizes human OBM and human sOBM, and is able to measure a very small amount of human OBM or human sOBM at a quantitative limit of~~. The measurement sensitivity thereof was about 1.25 ~~X 10~~$^{-3}$ to 2.5 X 10^{-3} pmol/ml (about 50- to 100 pg/ml for human OBM ~~with~~having a molecular weight of about 40 kDa, about 40- to 80 pg/ml for human sOBM ~~with~~having a molecular weight of about 32 kDa), and very small amounts of human OBM and human sOBM could be measured by the ELIZA. ~~The hybridomas which produce~~Hybridomas producing these two types of anti-human OBM/sOBM monoclonal antibodies, H-OBM 1 and H-OBM 4~~4~~, were named H-OBM1 and H-OBM4, respectively. ~~The~~Further, a hybridoma producing H-OBM 9, the anti-human OBM/sOBM monoclonal antibody ~~(H-OBM 9)~~ which ~~recognizes~~recognized both mouse OBM and mouse sOBM and ~~also has an osteoclast formation~~ exhibited osteoclastogenesis inhibitory activity, was named H-OBM9. These hybridomas were deposited with the National Institute of Bioscience and Human ~~-~~Technology~~,~~ of the Agency of Industrial Science and Technology~~, on November 5, 1993~~ of the Ministry of International Trade and Industry with ~~D~~deposit~~ion~~ ~~Nos.~~numbers FERM BP-6264 (H-OBM 1), FERM BP-6265 (H-OBM 4)~~,~~ and FERM BP-6266 (H-OBM 9)~~.~~ on November 5, 1997.

~~<~~[Example 37~~>~~]

Measurements of ~~m~~Mouse OBM and ~~m~~Mouse sOBM ~~u~~Using ~~a~~Anti-~~h~~Human OBM/sOBM ~~monoclonal antibody which recognizes mouse~~Monoclonal Antibody Recognizing Mouse OBM and ~~m~~Mouse sOBM

~~A sandwich ELISA was constructed~~Sandwich ELISAs using the anti-human OBM/sOBM monoclonal antibody~~,~~ H-OBM~~9, which recognizes~~9 recognizing mouse OBM and mouse sOBM and obtained in Examples 33 and 35 as ~~an~~ a solid -phased antibody ~~in~~, and using the ~~Examples 33 and 35, and the~~ anti-mouse OBM/sOBM polyclonal antibody obtained in Example 28 as an enzyme- labeled antibody ~~obtained in the example 28.~~, were constructed. ~~The mouse~~ Mouse OBM and ~~mouse sOBM were respectively~~ mouse sOBM were diluted stepwise with the ~~first~~ primary reaction buffer ~~to give various concentrations~~ in the same manner as in ~~the Example 35 and then measured sOBM according to the method described in the~~Example 35, and the mouse OBM and mouse sOBM were detected in the same manner as in Example 36. The results are shown in ~~Figure~~Fig. 28. As a result, it was ~~found~~confirmed that the mouse OBM and mouse sOBM ~~can~~could be ~~similarly measured using H-OBM 9 which is the~~ detected equally by use of the anti-human OBM/sOBM monoclonal antibody ~~recognizing~~ H-OBM 9 which recognized the mouse OBM and mouse sOBM ~~of the present invention~~. As shown ~~by~~ in the results of Example 35, ~~this anti-human OBM/sOBM monoclonal~~the antibody H-OBM 9 has~~d~~ a high dissociation constant ~~relative~~with respect to the mouse sOBM; in other words, ~~namely it has a comparatively~~the antibody had relatively low affinity ~~to~~for the mouse sOBM. ~~The sensitivity in~~Thus, the measurement sensitivities of mouse OBM (molecular weight~~,~~: about 40 kDa) and mouse sOBM (molecular weight~~,~~: about 32 kDa) by ~~this~~the above ELISA ~~assay was~~were about 25 ~~*~~X 10^{-3} pmol/ml (about 1 ng/ml for mouse OBM ~~and~~, about 0.8 ng/ml for mouse sOBM).

~~<~~[Example 38~~>~~]

Assay for Osteoclastogenesis- ~~i~~Inhibitory ~~a~~Activity of ~~a~~Anti-OBM/sOBM ~~a~~Antibody

It is known that an osteoclast-like cells (OCL) ~~are~~is ~~induced~~derived by co-culture of mouse spleen cells and ST2 cells (mouse bone marrow- derived ~~stromal cells;~~ interstitial cell) (Endocrinology, 125, ~~1805-1813~~1,805 to 1,813 (1989)). ~~Capability~~Thus, it was examined whether derivation of ~~the anti-~~ OCL was inhibited by addition of an OBM/ sOBM antibody ~~to inhibit the OCL formation when added~~ to the co-culture ~~system was studied~~. ~~Because the~~Since mouse OBM ~~is~~was expressed in this co-culture system, ~~a~~antibodies used in this Example were H-OBM 9 and rabbit anti-mouse OBM/sOBM polyclonal antibody ~~which recognizes~~recognizing mouse OBM ~~and an anti-human~~. The

OBM~~/sOBM monoclonal antibody (H-OBM-9) which recognizes both human OBM and mouse OBM antigens were used as the~~ antibodies ~~in this example. Seven hundred microliters per well of~~ each ~~anti-OBM antibody~~were diluted ~~serially~~stepwise with ~~α-~~αMEM containing 10% FCS and ~~350 μ~~added to a 24 well plate (Nunc Co., Ltd.) in an amount of 700 μl/well ~~of~~, and male mouse ~~splenocytes~~spleen cells suspended in the above medium (2 ~~×~~X 10^6 /ml) ~~suspended in the same medium described above~~ were also added ~~to each well~~ in ~~a 24~~ an amount of 350 μl/well ~~plate (Nunc)~~. ~~Next~~Then, ~~ST2 cells~~ trypsinized ~~and~~ST2 cells were suspended (8 X 10^4 cells/ml) in the above ~~mentioned culture~~ medium containing 4 ~~×~~X 10^{-8} M ~~V~~vitamin D_3 and 4 ~~×~~X 10^{-7} M ~~Dexamethazone (8 x 10^4 cells/ml) were~~dexamethasone, and the resulting suspension was added ~~to each well~~ in ~~the~~an amount of 350 ~~μ~~μl/well~~, followed by culturing for six~~. The plate was incubated at 37°C for 6 days ~~at~~for ~~37°C~~culture. After the plates ~~were~~was washed ~~once~~ with PBS once, the cells ~~in each well~~ were fixed ~~with a~~by mixture of 50% ethanol and 50% acetone ~~(50:50) for one hour~~ at room temperature for a minute. ~~The plates were~~ After the plate was air-dried ~~in air~~, ~~and 500 μl of~~ substrate solution was added ~~to each~~ in an amount of 500 μl/well ~~according to the~~in accordance with a protocol of ~~the LEUKOCYTE ACID PHOSPHATASE~~a leukocyte acid phosphatase kit (Sigma Co., Ltd.), ~~followed by incubating~~and the plate was left to stand at 37°C for 55 minutes ~~at 37°C~~so as to cause reaction. ~~Only the cells exhibiting the~~By this reaction, cell showing tartaric acid- resistant acid phosphatase activity (TRAP activity), which is a specific marker ~~for~~of osteoclasts, were stained ~~by this reaction~~. ~~The plates were~~After the plate was washed ~~once~~ with distilled water once and air-dried ~~in air, and~~ the number of TRAP-positive cells ~~was~~were counted. The results are shown in Table 4. As ~~shown in Table 4,~~a result, it was found that both of the rabbit anti-mouse OBM/sOBM polyclonal antibody and ~~the anti-human OBM/sOBM monoclonal antibody, H-OBM 9, which recognizes mouse OBM~~9 inhibited derivation of OCL ~~formation in a dose-dependent manner~~depending on the concentrations of the antibody. ~~These~~It was found that these antibodies ~~were found to possess~~had osteoclastogenesis- inhibitory activity ~~like~~as in the case of an osteoclastogenesis-~~inhibitory~~ factor, OCIF/OPG, and ~~thus are promising~~were useful as a ~~therapeutic agent~~medicament for treating bone metabolism abnormality ~~symptoms~~.

~~TABLE 4~~

Table 4

~~Amount of antibody (μg/ml)~~	~~Number of TRAP-positive multinucleates~~	
	~~Rabbit anti-mouse OBM/sOBM polyclonal antibody~~	~~Mouse anti-human OBM/sOBM monoclonal antibody (H-OBM 9)~~
~~0~~	~~1155±53~~	~~1050±45~~
~~10~~	~~510±24~~	~~650±25~~
~~100~~	~~10±3~~	~~15±4~~

Amount of Antibody Added (ng/ml)	Number of TRAP Positive Multinucleate Cells	
	Rabbit Anti-Mouse OBM/sOBM Polyclonal Antibody	Mouse Anti-Human OBM/sOBM monoclonal antibody (H-OBM 9)
0	1,155 ± 53	1,050 ± 45
10	510 ± 24	650 ± 25
100	10 ± 3	15 ± 4

(~~A~~average ~~±~~± standard deviation, n = 3)

~~<~~[Example 39~~>~~]

~~Human osteoclast formation-inducing activity of Trx-OBM~~

Osteoclastoginesis Inducing Activity of Trx-OBM

Mononuclear cells were prepared from whole blood ~~collected~~sampled from ~~the~~a vein of a ~~healthy~~normal adult ~~by density gradient~~human using Histopaque (Sigma Co., Ltd.) ~~according to the protocol~~with density gradient technique in accordance with an attached ~~thereto~~protocol. The mononuclear cells were suspended at a ~~cell density~~concentration of 1.3 ~~×~~X 10^6cells/ml ~~in~~with ~~α~~α-MEM containing 10^{-7} M ~~D~~dexamethasone, 200 ng/ml of macrophage colony stimulating factor (~~The Green Cross Corp~~Midori Juji Co., Ltd.), 10% fetal bovine serum~~,~~ and stepwise concentration (0 to 100 ng/ml) of purified Trx-OBM ~~(0-100 ng/ml)~~ obtained in Example 15. The ~~cell~~ suspension was added to ~~each well in~~a 48~~-~~well plates in ~~the~~an amount of 300 ~~μ~~μl ~~per~~/well, and the ~~cells were cultured~~plate was incubated at 37°C for ~~three~~3 days for culturing cells. ~~After~~Thereafter, the ~~culture broth~~medium was replaced with ~~the~~new (identical with above~~-mentioned culture medium~~), and the ~~cells were cultured~~plate was incubated at 37°C for ~~four~~another 4 days~~. The cultured~~ for culturing cells ~~having~~. Cell showing tartaric acid resistant acid phosphatase activity (TRAP activity) were selectively stained ~~according to~~by the method described in Example ~~5. The~~5, and the number of stained

~~multinucleates~~multinuclear cells was ~~measured by~~counted under the microscope ~~observation~~. The results are shown in ~~Figure~~Fig. 29. ~~It was confirmed that TRAP-positive multinucleates were induced in~~As a ~~dose dependent manner by addition of Trx-OBM~~result, ~~while no TRAP-positive~~ cells showing TRAP activity were hardly detected in the wells ~~to which~~containing no Trx-OBM, while TRAP positive multinuclear cells appeared in a manner depend on concentration of Trx-OBM when Trx-OBM was ~~not~~ added. ~~Moreover~~Further, these TRAP- positive multinucleates~~r were found~~ cells showed positive ~~to~~result for vitronectin receptor which is a marker ~~for~~of osteoclasts. ~~Furthermore~~In addition, when ~~similar~~the ~~cell~~same culture ~~was carried out~~conditions were used on ~~ivory~~dentin ~~slices~~fragments placed on ~~each well in~~ a 48-well plate, ~~pit formation was observed on the ivory slices~~absorption cavities were formed on the surface of dentin fragments only in the presence of Trx-OBM. ~~Based on these findings, Trx-OBM was formed to have the~~Thereby, it was revealed that Trx-OBM had activity ~~of inducing human osteoclast~~to induce formation of human osteoclasts.

<~~[~~Example 40~~>~~]

~~Inhibition of bone resorbing activity by anti-OBM/sOBM antibody~~

Bone Resorption Inhibitory Activity of Anti-OBM/sOBM Antibody

15-day pregnant ddy mice (Nippon SLC Co., Ltd.), 25 μCi of [^{45}Ca] -$CaCl_2$ solution (Amersham Co.~~) was~~, Ltd.) were injected subcutaneously ~~injected to ddY mouse (Japan SLC Co.) in the 15th day of pregnancy at a dose of 25 μCi per mouse to label the bone of the fetus~~, and fetal bones were labeled with ^{45}Ca. ~~Next~~On the following day, the ~~mouse was sacrificed~~mice were slaughtered, and their abdomens were opened to ~~obtain~~remove fetuses from the ~~fetus~~uteruses. ~~The forefoot of the fetus was drawn and~~A forelimb was removed from the fetus, the skin and muscle were removed to ~~obtain the~~take out a long ~~bones. The~~bone, and a cartilage on the long bone was also removed so as to ~~obtain~~leave only the ~~shafts~~diaphysis of the long bones. ~~The shafts of long bones were~~Each diaphysis was floated ~~one by one~~ in 0.5 ml of culture medium (BGJb medium (~~GIBCO BRL company~~Gibco Co., Ltd.) containing ~~a~~ 0.2% bovine serum albumin (Sigma Co., Ltd.)) ~~in each well in 24-well plates,~~ and cultured ~~for 24 hours~~ at 37°C in the presence of 5% CO_{2}~~-~~$_2$ for 24 hours. After completion of the pre-~~cultivation~~culture, the ~~bones were~~long bone was transferred ~~into various fresh~~ to a new culture medi~~a~~um (0.5

ml)~~, each~~ containing ~~one of different~~ various bone resor~~b~~ption~~g~~ factors (vitamin D_3, prostaglandins E_2, parathyroid hormone, interleukin 1-~~e~~α)~~,~~ and normal rabbit IgG (100 ~~μ~~μg/ml; as a control)~~,~~ or the rabbit anti-OBM/sOBM polyclonal antibody ~~prepared~~obtained in Example 28, ~~followed by further cultivation~~and then cultured for another 72 hours. After completion of the ~~cultivation~~culture, the long bones ~~were placed in~~ was put into 0.5 ml of ~~an aqueous solution of~~ 5% trichloroacetic acid aqueous solution (Wako Pure Chemicals ~~Co.~~Industries, Ltd.)~~,~~ and ~~allowed to stand~~treated at room temperature for ~~more~~at ~~than~~least 3 hours so as to ~~decalcify~~be decalcified. ~~Five~~To the conditioned medium and the trichloroacetic acid extract (0.5 ml each), 5 ml of ~~a~~ scintillator (AQUASOL-2, ~~PACKARD~~Packard Co., Ltd.) was added ~~to the culture broth and the extract of the trichloroacetic acid solution (each 0.5 ml) to measure~~ the radioactivity of ^{45}Ca~~, whereby the ratio~~ was measured. The proportion of ~~the~~ ^{45}Ca ~~which was~~ liberated ~~into~~in the culture ~~broth by~~solution due to bone resorption was calculated. The results are shown in ~~Figures~~Figs. 30 to 33. As a result, although the vitamin D_3 (10^{-8} M) ~~was found to~~caused increase ~~the~~of bone resor~~b~~ption~~g~~ activity, ~~but~~the bone resorption caused by the vitamin D_3 was inhibited by addition of the rabbit anti-OBM/sOBM polyclonal antibody ~~suppressed the bone resorption stimulated by vitamin D_3~~ in a concentration-dependent manner, and the bone resorption was completely ~~inhibiting~~inhibited by addition of the ~~increased bone resorption~~antibody at a concentration of 100 ~~μ~~μg/ml (~~Figure 30). Prostaglandins~~Fig. 30). Further, although bone resorption activity was increased in the presence of prostaglandin E_2 (10^{-6} M) ~~and~~or the parathyroid hormone (100 ng/ml) ~~also increased the bone resorbing activity. However~~, the bone resorption caused by the prostaglandin E_2 or the parathyroid hormone was almost completely inhibited by the addition of ~~100 μg/ml of~~ the rabbit anti-OBM/sOBM polyclonal antibody ~~almost completely inhibited the bone resorption stimulated by prostaglandins E_2 and parathyroid hormone~~ (~~Figures~~100 μg/ml)(Figs. 31 and 32). ~~On~~Meanwhile, the ~~other hand~~, normal rabbit IgG (100 ~~μ~~μg/ml)~~, which was~~ used as a positive control~~, did not affect the bone resorbing activity induced by prostaglandins E_2 and~~ had no effects on the bone resorption by the prostaglandin E_2 and the parathyroid hormone. ~~Bone~~Further, although bone resorption was ~~also increased by~~induced by the interleukin 1-~~e~~α (10 ng/ml)~~, but~~ as well, the bone resorption was inhibited significantly

~~inhibited~~ by the ~~addition of~~ rabbit anti-OBM/sOBM polyclonal antibody (100 ~~μ~~μg/ml) (~~Figure~~Fig. 23). ~~Based on~~From these results, it ~~is~~was ~~clear~~revealed that the antibody of the present invention ~~is~~was excellent as a ~~superior~~bone resorption inhibitory substance ~~as a bone resorption inhibitor~~. ~~The results obtained by similar~~As a result of conducting the same experiment ~~using~~on H-OBM 9 which ~~is~~was a mouse anti-human OBM/sOBM antibody, it was confirmed that ~~this antibody exhibits an almost~~ H-OBM 9 had approximately equi~~val~~ent bone resorption- inhibitory ~~effect as~~activity to that of the rabbit anti-OBM/sOBM polyclonal antibody.

Industrial Applicability

The present invention provides a novel protein ~~that specifically~~which binds ~~to~~ osteoclastogenesis- inhibitory factor (OCIF), a ~~process~~method for ~~preparing the protein~~production thereof, a method for screening ~~method for~~ a substance which controls expression of thise protein ~~using this~~by use of the protein, a ~~screening~~ method for screening a substance which inhibits or mod~~ulat~~ifies ~~the~~an activity of thise protein, a ~~screening~~ method for ~~the~~screening a receptor which binds the protein and transmits ~~the~~an activity ~~of this protein by binding thereto~~thereof, a pharmaceutical composition ~~which contains the~~comprising a substance obtained by ~~these~~said method for screening ~~methods~~, an antibody ~~for~~to the ~~said~~ protein, and an agent for treating bone metabolism abnormality which is formulated using the antibody.

~~Moreover, the present invention provides a DNA encoding~~Furthermore, the present invention provides a DNA which encodes a novel protein (OCIF- binding molecule) which binds ~~to~~ osteoclastogenesis- inhibitory factor (OCIF), a protein ~~which possesses~~having an amino acid sequence encoded by the DNA, a ~~method for preparing the~~ method for genetically producing a protein which specifically ~~binding to~~ binds to the OCIF ~~using said~~by use of the DNA ~~by a genetic engineering technique~~, and an agent for treating bone metabolism comprising ~~said~~the protein ~~for treating bone metabolism acatastasia~~. ~~Furthermore, the present invention provides a~~ Moreover, methods are provided for screening ~~method for~~ a substance which controls the expression of the OCIF- binding molecule, a ~~screening~~ method for screening a substance which binds to the OCIF binding molecule and inhibits or mod~~ulat~~ifies ~~the~~an activity ~~of the OCIF-~~

~~binding molecule by binding thereto~~thereof, a ~~screening~~ method for ~~the~~screening a receptor which binds OCIF binding molecule and transmits ~~the~~an activity ~~of the OCIF binding molecule by binding thereto~~thereof, and a pharmaceutical composition ~~which contains the~~comprising a substance obtained by ~~these~~said method for screening ~~methods~~.

~~Still further, the present invention provides a DNA encoding~~Also provided is: DNA, which encodes a novel human protein ~~capable of binding to osteoclastogenesis inhibitory factor, OCIF~~ (human-derived OCIF- binding molecule, human OBM) which binds osteoclastogenesis inhibitory factor (OCIF), a protein ~~containing~~and having an amino acid sequence encoded by the DNA, a ~~process~~method for ~~preparing~~genetically producing a protein ~~having characteristics of~~which specifically ~~binding to~~binds OCIF and ~~exhibiting~~has a biological activity to support and promote the ~~osteoclast~~ differentiation and maturation of osteoclasts by ~~means~~use of ~~genetic engineering technique~~the DNA, and an agent for treating bone metabolism abnormality ~~using~~comprising the protein.

~~Furthermore, the present invention provides~~

Also provided are: a ~~screening~~ method for screening a substance which controls expression of the OCIF- binding molecule, a ~~screening~~ method for screening a substance which binds the OCIF binding molecule and inhibits or mod~~ulat~~ifies ~~the~~an activity ~~of the OCIF binding molecule by binding thereto~~thereof, a ~~screening~~ method for ~~the~~screening a receptor which binds the OCIF binding molecule and transmits the biological activity ~~of the OCIF- binding molecule by binding thereto~~thereof, and a pharmaceutical composition ~~which contains the~~comprising a substance obtained by ~~these~~said method for screening ~~methods~~, as well as an antibody to the human-derived OCIF- binding protein, and an agent for preventing and/or treating bone metabolism abnormality ~~symptoms~~which is formulated using the antibody.

~~In addition~~Moreover, the present invention provides ~~antibodies~~an antibody (anti-OBM/sOBM antibody) which recognizes both of the following antigens ~~(anti-OBM/sOBM antibodies)~~, ~~one is~~i.e., a membrane ~~bound protein~~ binding molecule (OCIF binding molecule; OBM) which specifically binds to an OCIF ~~(OCIF binding molecule; OBM)~~, and ~~the other~~ a soluble OBM (sOBM) ~~which does not have a~~lacking membrane binding ~~region~~sites, a ~~process~~method for ~~preparing~~production of the anti~~gen~~body, a method for measuring the OBM and sOBM ~~using these antibodies~~by use of the antibody,

and an agent for preventing and/or treating bone metabolism abnormality ~~symptoms~~which ~~using~~comprise the antibody as an ~~effective~~active ~~component~~ingredient.

The proteins ~~and~~ or antibod~~y~~ies pre~~par~~sented by ~~the process of~~ the present invention are useful as ~~medicines and/or~~medicaments, experimental reagents or diagnostic reagents ~~for research and test purposes~~.

~~Description of deposited microorganisms~~

~~(1) Name and address of the depository organization to which microorganism was deposited~~

Reference to Deposited Microorganisms

(1) Name and Address of Depository Institution:

National Institute of Bioscience and Human-Technology of the Agency of Industrial Science and Technology of the Ministry of International Trade and Industry

1-~~3,~~1-3 Higashi~~-1-Chome~~, Tsukuba-shi, Ibarak~~g~~i-ken, Japan(~~postal code~~(zip: 305)

Date of ~~deposition to the depository organization~~Deposit:

May 23, 1997

~~The deposition number~~

Deposit Number:

FERM BP-5953

~~(2) Name and address of the depository organization to which microorganism was deposited~~

(2) Name and Address of Depository Institution:

National Institute of Bioscience and Human-Technology of the Agency of Industrial Science and Technology of the Ministry of International Trade and Industry

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Date of ~~deposition to the depository organization~~Deposit:

August 13, 1997

~~The deposition number~~

Deposit Number:

FERM BP-6058

~~(3) Name and address of the depository organization to which microorganism was deposited~~

(3) Name and Address of Depository Institution:

National Institute of Bioscience and Human-Technology of the Agency of Industrial Science and Technology of the Ministry of International Trade and Industry

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Date of ~~deposition to the depository organization~~Deposit:

November 5, 1997~~-(Original deposition date)~~

~~The deposition number~~

Deposit Number:

FERM BP-6264

~~(4) Name and address of the depository organization to which microorganism was deposited~~

(4) Name and Address of Depository Institution:

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Date of ~~deposition to the depository organization~~Deposit:

November 5, 1997~~-(Original deposition date)~~

~~The deposition number~~

Deposit Number:

FE~~P~~RM BP-6265

~~(5) Name and address of the depository organization to which microorganism was deposited~~

(5) Name and Address of Depository Institution:

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Date of ~~deposition to the depository organization~~Deposit:

November 5, 1997 ~~(Original deposition date)~~

~~The deposition number~~

Deposit Number:

FERM BP-6266